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# Optimisation of the Post-Harvest Conditions to Produce Chocolate Aroma from Jackfruit Seeds

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1 ABSTRACT

2 Jackfruit seeds are an under-utilized waste in many tropical countries. In this work,  
3 we demonstrate the potential of roasted jackfruit seeds to develop chocolate  
4 aroma. Twenty-seven different roasted jackfruit seed flours were produced from  
5 local jackfruit by acidifying or fermenting the seeds prior to drying, and roasting  
6 under different time/temperature combinations. The chocolate aroma of groups of  
7 four flours were ranked by a sensory panel (n=162) and response surface  
8 methodology was used to identify optimum conditions. The results indicated a  
9 significant and positive influence of fermentation and acidification on the  
10 production of chocolate aroma. SPME/GC-MS of the flours showed that important  
11 aroma compounds such as 2,3-diethyl-5-methylpyrazine and 2-phenylethyl acetate  
12 were substantially higher in the fermented product, and that the more severe  
13 roasting conditions produced 2-3 times more 2,3-diethyl-5-methylpyrazine, but less  
14 3-methylbutanal. Moisture,  $a_w$ , pH, luminosity and color were also monitored to  
15 ensure that these properties were similar to cocoa powder or cocoa substitutes.

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24 Keywords: jackfruit seeds, chocolate aroma, waste utilization, sensory analysis,  
25 SPME/GC-MS

26 INTRODUCTION

27 Jackfruit (*Artocarpus heterophyllus* Lam.) is a large tropical fruit which is  
28 abundant in South America, Asia, Africa and Australia. It is a fleshy compound fruit  
29 (syncarp) belonging to the Moraceae family and takes 3-6 months to reach ripeness.  
30 The fruit weight ranges from 2 to 36 kg and its seeds account for around 15-18 % of  
31 the total weight of the fruit.<sup>1,2</sup> Generally the seeds are boiled, steamed and roasted  
32 before eating, providing a cheap source of fiber, protein and minerals. In many  
33 countries, including Brazil, jackfruit seeds are an under-utilized waste stream.

34 There are several publications reporting the use of waste jackfruit seeds to  
35 produce starch,<sup>3-6</sup> but there is little reported in the literature on their potential to  
36 generate flavor. For the first time we found that after roasting, jackfruit seeds  
37 imparted an aroma similar to chocolate. Chocolate aroma has been well-  
38 characterized<sup>7,8</sup> and a number of different aroma compounds have been found to  
39 contribute to the complex and characteristic aroma of chocolate. The most odor-active  
40 compounds in milk chocolate identified by Schnermann and Schieberle<sup>7</sup> include 3-  
41 methylbutanal, phenylacetaldehyde and 2,3-diethyl-5-methylpyrazine and, a few more  
42 in roasted cocoa beans.<sup>9</sup> Some pyrazines have been shown to contribute significantly  
43 to the unique flavor of roast and toast foods<sup>9</sup> and are used to determine the quantity  
44 and quality of cocoa flavor.<sup>10</sup> They impart chocolate, cocoa, hazelnut, roasted, coffee,  
45 earth and green aromas.<sup>11,12</sup> As with cocoa, the post-harvest pre-treatments and  
46 roasting of the jackfruit seeds are likely to influence the formation of these compounds  
47 and the quality of the aroma.

48 All three stages of the process (fermentation, drying and roasting) can have an  
49 influence on the final pyrazine concentration. During fermentation, enzymatic and  
50 microbial processes induce physical and chemical changes in seeds which result in

51 browning reactions.<sup>13</sup> Some volatile compounds are formed at this stage, as well as  
52 free amino acids and sugars which are substrates for the subsequent flavor-forming  
53 reactions<sup>14</sup> which take place during roasting. The influence of fermentation parameters  
54 on the aroma of roasted cocoa beans is well understood and has been reviewed  
55 recently.<sup>15</sup> Kirchhoff et al.<sup>15</sup> demonstrated that chocolate aroma was correlated to  
56 proteolysis and the subsequent accumulation of free amino acids. The proteolytic  
57 enzymes such as endopeptidases and proteases are highly sensitive to pH, so pH  
58 control is important during cocoa fermentation to regulate the activity of different  
59 enzymes. These products of fermentation (amino acids and reducing sugars) are the  
60 precursors of pyrazines which are formed during roasting in the Maillard reaction.<sup>16-19</sup>

61 Cocoa (*Theobroma cacao*) is a culture which is highly sensitive to changes in  
62 climate, is susceptible to many typical diseases and local farmers struggle to compete  
63 with international cocoa suppliers.<sup>20,21</sup> Global cocoa production is around 3.7 million  
64 tons and this is not expected to grow significantly in the next 10 years,<sup>22</sup> however  
65 demand by 2020 is estimated to be 4.5 million tons.<sup>21</sup> In this context, new sources of  
66 chocolate aroma and flavor are important to meet the increase in demand and provide  
67 alternative revenue streams for local farmers and communities in Brazil.

68 The aim of this work is optimize the production of chocolate aroma from  
69 jackfruit seeds by treating them under conditions similar to those used in the cocoa  
70 process. Seeds will be acidified or fermented prior to drying, and roasted under  
71 different time/temperature combinations. Sensory ranking tests will be used to  
72 assess the chocolate aroma and key aroma compounds will be analysed by  
73 SPME/GC-MS.

74 MATERIALS AND METHODS

75           **Chemicals.** Standards of 3-methylbutanal, phenylacetaldehyde, 2-phenylethyl  
76 acetate, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-  
77 dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, 2,3-diethyl-5-  
78 methylpyrazine, 1,2-dichlorobenzene and the alkane standards C<sub>6</sub>–C<sub>25</sub> were purchased  
79 from Sigma Aldrich Química, São Paulo, Brasil.

80           **Jackfruit.** Twenty five jackfruit of the hard pulp varieties were manually collected  
81 from one single tree, between October 2013 and January 2014, in the countryside of  
82 São Paulo, Brazil, selecting fruit of similar size ( $5 \pm 1$  kg) and ripeness, as indicated by  
83 the yellow color of the shell. Jackfruits were cleaned manually in running water, the  
84 seeds removed and the pulp discarded. These seeds were subjected to one of three  
85 different treatments prior to roasting, producing either dried jackfruit seeds (DJS),  
86 acidified jackfruit seeds (AJS) or fermented jackfruit seeds (FJS). For each treatment,  
87 the seeds from 7-9 jackfruit were pooled, and treated and dried in four  $\times$  1.5 kg  
88 batches (3 kg batch for FJS) as described below. The dried beans (50 g from each of the  
89 four batches) were roasted in 200 g portions. In total, 11 bags of roasted flour (200 g)  
90 were prepared for each of the three treatments.

91           **Seed processing.** For the dried jackfruit seed (DJS), the seeds were dried in an  
92 oven at 60 °C with air circulation. After 24 h, the spermoderms were manually  
93 removed, and the seeds remained for a further 24 h in the same oven at the same  
94 temperature.

95           For the acidified jackfruit seeds (AJS), treatment was carried out at ambient  
96 temperature ( $25 \pm 3$  °C). For each batch, the seeds (1.5 kg) were placed in polyethylene  
97 trays (28 x 42 x 7.5 cm) with 1% w/w acetic acid in potable water (3 kg). After five days  
98 the solution was removed and the seeds were dried using the same method as for DJS  
99 (2  $\times$  24 h).

100 For the fermented jackfruit seeds (FJS), simulating what is done with cocoa,  
101 seeds (3 kg) were placed in polyethylene boxes to ferment with added jackfruit pulp  
102 (1.5 kg), perianth (0.52 kg), and banana leaves (0.1 kg) as a source of yeast. For the first  
103 6-7 days of fermentation, the boxes were closed to promote anaerobic fermentation  
104 but, for the remaining 7-8 days, the boxes were opened and the fermenting mass was  
105 rolled daily to promote oxidation. The seeds were removed and dried using the same  
106 method as for DJS (2 × 24 h). These processes are summarized in Figure 1A. The yield  
107 from each treatment was expressed as in equation 1.

108 
$$\text{Yield (\%)} = (\text{weight of flour after drying}) \times 100 / \text{weight of raw jackfruit seeds} \quad \text{Eqn1}$$

109 During acidification and fermentation, the ambient temperature and the  
110 temperature of the fermenting mass were measured every 24 h<sup>23</sup> (AOAC Methods  
111 13.010; 32.010; 32.016 and 32.017).. For AJS, the pH of an aliquot of liquid extracted  
112 from the mass in the polyethylene boxes was measured directly. For the FJS mass, 10 g  
113 of the fermenting mass was added to 100 mL of distilled water. In both cases, the pH  
114 was measured using a pH meter with a glass electrode standardized at the experiment  
115 temperatures over the range from 7.0 to 4.0. For FJS, total titratable acidity (AOAC  
116 945.08)<sup>24</sup> was measured using 5 g fermenting mass, diluted 10 times and filtered.  
117 Proximate analysis was carried out according to Horwitz et al.<sup>23</sup>

118 **Roasting and Grinding.** For each treatment, 11 batches of seeds (200 g) were  
119 roasted in a rotary electric oven (Probat<sup>®</sup> laboratory sample roaster, Emmerich am  
120 Rhein, Germany) with digital temperature control, using conditions defined by the  
121 response surface methodology. A central composite design was used for each  
122 treatment (Figure 1B). Two factors (roasting time and temperature) were each tested  
123 at five levels, with three repetitions of the central point totaling 11 samples. However,  
124 preliminary experiments showed it was necessary to select different roasting



125 conditions for each treatment to avoid burning of the FJS yet achieve significant  
126 roasting in the AJS. The temperature ranged from 150 to 201°C ± 0.1°C and the  
127 roasting time from 33 to 47 min. The roasted seeds were then milled in a hammer mill  
128 to produce a “flour”. There was no heating of the sample during milling, minimizing  
129 loss of volatile compounds at this stage. Flours were packed under vacuum and stored  
130 without light at 8±1 °C.

131 **Analysis of Flours.** Water activity was determined from the temperature of the  
132 dew point (Aqualab®), moisture was determined by a standard gravimetric method,  
133 and color was measured instrumentally using a Minolta® colorimeter, with illuminant C,  
134 previously calibrated with a white surface (Y = 93.7, x = 0.3135 and y = 0.3195) based  
135 on the CIE-lab L,\* a,\* b,\* scale. The pH was determined in triplicate using 2 g of flour  
136 added to distilled water (20 mL). The quality of the chocolate aroma was based on a  
137 sensory comparison and the relative concentration of selected aroma compounds was  
138 measured by GC-MS. The proximate composition was only carried out on flours with  
139 the highest sensory rankings.

140 **Sensory analysis.** All sensory evaluations were approved by the Ethics  
141 Committee of Human Research of the ESALQ/USP (COET/077/131).

142 **Preliminary sensory tests.** Preliminary tests were carried out to determine the  
143 optimum temperature and time for sample exposure prior to the panelists receiving  
144 the sample for assessment. In this preliminary assay using AJS flour, the samples were  
145 placed in a water bath for five different combinations of time (30, 60, 120 s) and  
146 temperature (25, 36.5, 48 °C) prior to sniffing by a small panel comprising 21 untrained  
147 members aged 18-40 years (76% women). Each panelist was asked to rank groups of  
148 three samples in increasing order according to the intensity of the chocolate aroma

149 (Table 1). There was no significant difference between the conditions used to  
150 equilibrate the samples so the conditions were standardized at 40 °C for 120 s.

151 **Sensorial ranking test.** Ranking tests were used to determine the relative  
152 intensity of chocolate aroma in 11 samples for each treatment (DJS, AJS and FJS) using  
153 incomplete blocks (Figure 2). Each sample (3 g) was placed in an amber vial coded with  
154 a random three-digit number and, prior to sensory evaluation, the vial was heated for  
155 120 s in a water bath at 40 °C, these conditions having been selected from the  
156 preliminary tests. Panelists received simultaneously four coded samples to rank in  
157 increasing order of intensity of chocolate aroma, from least (=1) to most (=4). Total  
158 ranking scores were used, thus the higher score representing the greater chocolate  
159 aroma. The data obtained from the panelists were collected and analyzed using  
160 Compusense five.<sup>®</sup> At the end of the session, the panelists were asked to describe  
161 different aromas they identified in each of the samples using their own free choice of  
162 descriptors.

163 **Sensory experimental design.** Untrained panelists (162) aged 18-54 years (60%  
164 women) were randomly divided into three equal groups of 54 – one group for each  
165 treatment (DJS, AJS and FJS). In order to reduce the number of comparisons to be  
166 assessed by the panel, a balanced, incomplete block, design of experiment was used<sup>25</sup>  
167 to construct a second order model based on the 11 samples in the central composite  
168 design.<sup>25</sup> However, to minimize panelist fatigue, the central point was represented by a  
169 blend of the three central points (reducing the number of samples to 9) and the three  
170 central points were assessed by the same panelists in a second sensory session.

171 In the first sensory session panelists received four samples in a balanced  
172 incomplete block (Figure 2). Each sample block consisted of 18 comparisons (9 samples  
173 each appearing 8 times). The sample block was repeated three times (for 54 panelists)

174 and the parameters, as defined by Cochran and Cox<sup>24</sup>, were T=9; k=4; r=8; B=18; L=3;  
175 E=84; Z=3.

176 In the second sensory session, the same panelists received three samples of the  
177 central point (0, 0) (Figure 2). These samples were delivered at the same time, in a  
178 randomized and balanced complete block<sup>25</sup>. Total ranking scores from the second  
179 session were transformed to be comparable to the first sensory session. Thus it was  
180 possible to assess the variation between the central points and validate the response  
181 surface for intensity of chocolate aroma.

182 **Volatile analysis.** Jackfruit flour (DJS, AJS and FJS) (3 g) was placed in a 20 mL  
183 SPME vial with 1 $\mu$ L of 1,2-dichlorobenzene in methanol (130.6  $\mu$ g/mL) and vortexed for  
184 2 min. After equilibration at 45 °C for 15 min, the triple phase fiber (65  $\mu$ m  
185 PDMS/DVB/Carboxen from Supelco) was exposed (1cm) to the headspace above the  
186 sample for 55 min under magnetic agitation (635 rpm). These conditions had  
187 previously been optimized using surface response methodology.

188 The volatile compounds extracted by the fiber were analyzed by GC-MS using a  
189 Shimadzu<sup>®</sup> QP2010 GC-MS equipped with a RTX5MS column (30 m, 0.25 mm i.d., 0.25  
190  $\mu$ m film thickness). Volatile compounds were desorbed for 1 min during a splitless  
191 injection at 200 °C. During desorption, the oven was maintained at 40 °C for a further 8  
192 min, and then the temperature was raised at 4 °C/min to 200 °C, and then 10 °C/min to  
193 280 °C totaling 56 min. MS was carried out using 70 eV electron impact, and *m/z* were  
194 monitored in the range 40 to 500, in scan mode. Helium was the carrier gas and the  
195 flow rate was 1 mL/min in constant flow. A series of n-alkanes C<sub>6</sub>–C<sub>20</sub> was analyzed  
196 under the same conditions to obtain linear retention indices (LRIs) for comparison with  
197 authentic samples. All volatile compounds listed were identified by comparison of their

198 mass spectrum and LRI with that of an authentic standard run under similar conditions.

199 Each sample was analyzed three times.

200 Peak areas for 3-methylbutanal, phenylacetaldehyde and 2-phenylethyl acetate  
201 were measured using the total ion chromatogram. For the following compounds, the  
202 peak area was approximated using the area of a characteristic  $m/z$  which was  
203 multiplied by a factor calculated from the spectrum obtained from the authentic  
204 standard: 2-methylpyrazine  $m/z$  94, factor 3; 2,5/6-dimethylpyrazine (coelute)  $m/z$   
205 108, factor 2.5; 2,3-dimethylpyrazine  $m/z$  67, factor 4; trimethylpyrazine  $m/z$  81, factor  
206 14; tetramethylpyrazine  $m/z$  54, factor 5; 2,3-diethyl-5-methyl-pyrazine  $m/z$  121,  
207 factor 20. The approximate relative concentration of each compound was obtained by  
208 comparing the peak area against that of the internal standard (1,2-dichlorobenzene),  
209 using 1 as a response factor.

210 **Statistical analysis and response surface methodology.** The central composite  
211 design, Statistics<sup>®</sup> (2014), was selected for use. The two key responses were intensity of  
212 chocolate aroma, 3-methylbutanal and 2,3-diethyl-5-methylpyrazine concentration,  
213 although water activity, moisture, pH and color were also monitored. The two  
214 independent variables of the design, roasting time and temperature, were coded as  
215  $x$ , and  $y$ , respectively. Equation 2 shows the quadratic polynomial model that was  
216 fitted to each response, where  $b_0$ ,  $b_1$ ,  $b_2$ ,  $b_{11}$ ,  $b_{12}$  and  $b_{22}$  are the regression  
217 coefficients;  $x$  and  $y$  are the values of the independent variables for roasting time (min)  
218 and temperature (°C) respectively.

$$219 \quad z = b_0 + b_1x + b_2y + b_{11}x^2 + b_{22}y^2 + b_{12}xy \quad \text{Eqn 2}$$

220 The analysis of variance (ANOVA) tables were generated and regression  
221 coefficients of individual linear, quadratic and interaction terms were determined by  
222 using design expert software (Statistics<sup>®</sup>). The significances ( $p \leq 0.05$ ) of all terms in the

223 polynomial model were judged statistically by computing the F value. XLStat was used  
224 to carryout 2-way ANOVA on the volatile data and calculate Fisher's least significant  
225 difference at p=0.05.

## 226 RESULTS AND DISCUSSION

227 **Jackfruit seed processing.** In cocoa beans, control of the fermentation process is  
228 required because unfermented beans develop little chocolate flavor, and excessive  
229 fermentation may also result in unwanted flavors when roasted.<sup>26</sup> Generally for cocoa,  
230 fermentation lasts between 5 to 8 days, and the end point is determined by  
231 experience<sup>27</sup> based on reducing acid notes and maximizing chocolate flavor in the final  
232 roasted product.<sup>28-30</sup>

233 In this study, the fermentation process of 12 days was necessary, maybe because  
234 jackfruit seeds are bigger in comparison to cocoa beans and there is more substrate to  
235 ferment. During the fermentation it is important to kill the embryo at the beginning to  
236 ensure the success of the fermentation process and the formation of flavor  
237 compounds. Jinap and Dimick<sup>31</sup> and Heemskerk et al.<sup>32</sup> reported that a pH close to 4  
238 would destroy the embryo; in jackfruit we found this value around day 3-4 of  
239 fermentation (Figure 3A) whereas the acidification process started at pH 3 and  
240 fluctuated between pH 3 and pH 4 (Figure 3B). In cocoa, samples are considered well  
241 fermented at pH > 4 although this varies with variety.<sup>28</sup> In practice, an increase in pH of  
242 the seeds has been shown to improve chocolate flavor during fermentation and  
243 alkalization<sup>29-33</sup> reported that pH values lower than 4.5 in the seeds decreased the  
244 aromatic potential of the cocoa beans. So there is a balance between achieving a pH  
245 which is low enough to kill the embryo but high enough to form aroma compounds.

246 Although titratable acidity in FJS was very variable, the overall trend was for an  
247 increase as the pH dropped (Figure 3A and 3C). Rodriguez-Campos et al.<sup>34</sup> reported

248 similar results during cocoa fermentation with a correlation coefficient of -0.91  
249 between pH and titratable acidity, and -0.86 for the correlation of the concentration of  
250 acetic and lactic acid with pH.

251 Acetic and lactic acid are present in the first and second stages of cocoa  
252 fermentation, when anaerobic yeasts and lactic acid bacteria are present, respectively.  
253 Towards the end of fermentation, when aeration increases, the acetic acid bacteria  
254 become more significant. They are responsible for converting alcohol to acetic acid,  
255 and since this reaction is exothermic (Figure 3D), it is likely that they are also  
256 responsible for the increase in temperature of the fermenting jackfruit mass.<sup>32</sup> At the  
257 end of the jackfruit fermentation period (day 12), the temperature of the mass had  
258 risen from ambient to values near to 40 °C, similar to the rise during fermentation of  
259 cocoa beans although, in cocoa, temperatures can reach 45 °C.<sup>28</sup> Figure 3 shows the  
260 pH, titratable acidity and temperature profile for FJS and pH for AJS.

261 For such a natural and variable process, these figures show that, with the  
262 exception of titratable acidity, these processes are fairly reproducible. In addition, it  
263 shows that jackfruit seeds can be fermented and dried under similar, albeit slightly  
264 longer, conditions to those applied to cocoa beans, resulting in a similar drop in pH  
265 which in cocoa results in the formation of aroma precursors.

266 **Yield, pH, water activity ( $a_w$ ), moisture, luminosity ( $L^*$ ) and chroma ( $c^*$ ) of**  
267 **jackfruit seed flours.** In terms of total mass, the yields of flour obtained from DJS, AJS  
268 and FJS were 48%, 45% and 40% respectively.

269 The pH of the roasted jackfruit seed flours were highest (pH > 5) in the flours  
270 which had been roasted at the highest temperature (independent of seeds processing)  
271 and the lowest pH (< 4.9) was found in general in the FJS flour (Table 2). These pH  
272 values are similar to those reported in traditionally fermented and roasted cocoa (4.75

273 to 5.19).<sup>28,33-36</sup> In other cocoa substitutes, Yousif and Alghzawi<sup>37</sup> found roasted carob  
274 powder to be pH 4.81 and Queiroz and Garcia<sup>38</sup> reported the pH of roasted cupuaçu  
275 flour as 4.77 - both similar to fermented and roasted jackfruit seeds (Table 2).

276 The pH of the flours can be fitted to a 3-dimensional surface as a function of time  
277 and temperature by using a combination of linear and quadratic terms, as well as an  
278 interaction term, to construct a polynomial equation. The correlation coefficient ( $r^2$ )  
279 indicates how well the data fit the model, and the p-value associated with each  
280 coefficient in the equation indicates the certainty with which this term influences the  
281 response (Table S1). The correlation coefficient is good ( $r^2 > 0.7$ ) so it is possible to  
282 model and predict the pH of the flour from AJS and FJS as a function of time (x) and  
283 temperature (y) using equations 3 and 4 respectively. In FJS flour (Eqn 4), there was a  
284 linear and quadratic relationship with temperature ( $p = 0.006$  and  $0.03$  respectively)  
285 and a linear correlation with time ( $p = 0.04$ ). For AJS flour we found significant linear  
286 effects with temperature ( $p = 0.01$ ). However, for DJS flour, the final pH was relatively  
287 insensitive to changes in the roasting conditions and the model cannot be used  
288 predictively ( $r^2=0.6$ ). The pH was on average higher in flours from DJS compared to AJS  
289 and FJS.

290 
$$pH_{AJS}=37.455-0.42557x-0.27377y+0.001036x^2+0.000566y^2+0.0019444xy \quad (r^2=0.81)$$

291 Eqn 3

292 
$$pH_{FJS}=19.94+0.0899x+0.2223y-0.00159x^2+0.0006789y^2+0.0002987xy \quad (r^2=0.93) \quad \text{Eqn 4}$$

293 Generally the moisture was associated with water activity ( $a_w$ ) in flours, and both  
294 tended to decrease as roasting conditions became more severe (Table 2). In FJS flours,  
295 the highest roast temperature (180 °C) for 40 min (0, 1.41) produced the lowest  $a_w$  and  
296 the lowest moisture was obtained at 186-192 °C for a 35-40 min roast. In this study we  
297 found 2.3% moisture in flour from FJS at (0, 1.41) which was high compared to DJS and

298 AJS flours roasted under similar conditions. By comparison, Yousif and Alghzawi<sup>31</sup>  
299 found 9.0 and 2.5% moisture for roast carob powder (150 °C for 60 min) and cocoa  
300 powder respectively, and Queiroz and Garcia<sup>38</sup> showed 3.0% moisture in roasted  
301 cupuaçu powder. The  $a_w$  described for both these substitutes was around 0.4. Thus  
302 flours of jackfruit seeds have similar or lower moisture and  $a_w$  in comparison to cocoa  
303 and other substitutes, which is important to restrict microbial growth in the flours and  
304 for application in other products. The surface response design allows use of equations  
305 5, 6 and 7 ( $x$ = time and  $y$ = temperature) to predict the moisture in the flour of DJS, AJS  
306 and FJS ( $r^2 > 0.7$ ); in all equations we could observe the significant linear effect of both  
307 roasting time and temperature in determining final moisture content (Table S1).

308  $\text{Moisture}_{\text{DJS}} = 65.71 - 0.2845x - 0.6427y - 0.003497x^2 + 0.001512y^2 - 0.000383xy$  ( $r^2=0.97$ ;  
309 linear temperature effect  $p=0.001$ ) Eqn 5.

310  $\text{Moisture}_{\text{AJS}} = 39.15 - 0.349499x - 0.26448y - 0.0067x^2 + 0.0006943y^2 - 0.0012498xy$  ( $r^2=0.92$ ;  
311 linear temperature effect  $p=0.010$ ) Eqn 6.

312  $\text{Moisture}_{\text{FJS}} = 105.447 - 1.6209x - 0.7213y + 0.000926x^2 + 0.001246y^2 - 0.0050106xy$  ( $r^2=0.90$ ;  
313 linear temperature effect  $p=0.020$ ) Eqn 7.

314 In contrast,  $a_w$ , where there was much greater variability in the responses, can  
315 only be predicted in DJS flour and only the linear term in temperature was significant  
316 (equation 8), and negative, showing that as the temperature increased, the  $a_w$   
317 decreased.

318  $a_{w\text{DJS}} = -4.454 + 0.1165x + 0.03237y - 0.0011974x^2 - 0.000087113y^2 - 0.000127xy$   
319 ( $r^2=0.75$ ; linear temperature effect  $p=0.04$ ) Eqn 8.

320 Color in food is important because appearance can contribute to recognition,  
321 perception and enjoyment of the food. For substitutes, it is necessary to match the  
322 original product as closely as possible. In cocoa powder the luminosity ( $L^*$ ) is low (near



323 to black and brown), similar to the jackfruit flour which was produced from the high  
324 temperature roasts. L\* tended to be lower (darker) in FJS compared to AJS flour. For  
325 chroma, the results were the reverse with high intensity color (larger chroma value) in  
326 the higher roasts, and the FJS flours having the least intense color, although there  
327 were few significant differences between roasting treatments. Luminosity results for  
328 fermented jackfruit seeds were similar to values in roasted cupuaçu. Cohen and Jackix,  
329 <sup>39</sup> reported L\* of 42 in cupuaçu liquor compared to values of 50-70 found in the  
330 jackfruit. Sacchetti et al.<sup>40</sup> found L\* = 21 for roast cocoa beans (145 °C to 30 min) and  
331 Sengül et al.<sup>41</sup> found L\*=19. Only Gu et al.<sup>12</sup> had slightly higher luminosity (L\*= 41) for  
332 roast cocoa (160 °C for 30 min). Therefore depending on the kind of product  
333 developed using jackfruit seed flour, it may be necessary to modify the color with  
334 other ingredients. It is possible to predict the luminosity and chroma of DJS flour using  
335 equations 9 and 10 (x= time and y= temperature, r<sup>2</sup> > 0.7). In both equations we  
336 observed a significant negative linear effect (p ≤ 0.05) of roast temperature (i.e. as  
337 temperature increased, L\* decreased and the product became darker), and, for DJS,  
338 roast time was also significant. For acidified and fermented flours we found no  
339 significant effect of roasting conditions (r<sup>2</sup> was 0.60 and 0.51 for chroma; and for  
340 luminosity 0.44 and 0.52 for AJS and FJS respectively).

341  $L^*_{DJS} = 70.645 - 0.9291x - 0.2478y + 0.005709x^2 + 0.0000373y^2 + 0.0060888xy$  (r<sup>2</sup>=0.94; linear  
342 temperature effect p=0.007; linear time effect p=0.0002) Eqn 9

343  $Chroma_{DJS} = 19.39 + 0.27954x - 0.47968y - 0.008682x^2 - 0.00159939y^2 + 0.002884xy$   
344 (r<sup>2</sup>=0.88; linear temperature effect p=0.03) Eqn 10

345 **Proximate composition of jackfruit seed flours.** The proximate analysis was  
346 only carried out on the three best roast conditions determined by sensory score (Table  
347 3). For DJS flours, where there was no significant difference between the samples in

348 terms of sensory score, a sample with high pyrazine content and a high sensory score  
349 was selected. The different treatments produced different proximate composition. The  
350 moisture was smallest in AJS flours, maybe because during five days in acetic acid  
351 solution the seed had dehydrated. AJS and DJS were similar in proximate content. In  
352 FJS, the fermentation process results in the breakdown of carbohydrates and the  
353 release of CO<sub>2</sub>. This is reflected in the proximate analysis where the remainder of the  
354 material is assumed to be carbohydrate. This is significantly lower in FJS (53%)  
355 compared to DJS (65%) and AJS (73%) respectively. The indirect consequence of this is  
356 a small increase in the % contribution from the other analytes.

357       Moisture,  $a_w$ , pH and color of the roasted jackfruit flours tended to vary with the  
358 time and temperature of the roasting conditions. However, the pH and moisture of the  
359 milled flours were similar to those of cocoa powder, and although the color was a bit  
360 pale (high L\*), these flours have similar properties to cocoa, carob and cupuaçu  
361 powders, and could be used in similar products.

362       **Sensory assessment of chocolate aroma of jackfruit flours.** The response  
363 surfaces for the sensory ranking tests are shown in Figure 4 A-C and the data are  
364 shown in Table 4. The correlation coefficients for the 3D surface models for all three  
365 processes (dry, acidified and fermented) were  $\geq 0.7$ . For DJS flours (Figure 4A), there  
366 was no significant difference between samples ( $p \leq 0.05$ ) in the perception of sensory  
367 chocolate aroma, although the model showed a linear effect with temperature ( $p \leq$   
368 0.03) suggesting that the higher temperature may increase slightly the chocolate  
369 aroma. For AJS flours, roasting at the temperature of the central point (180 °C)  
370 generated the greatest sensory perception of chocolate aroma (Table 4). The model  
371 showed a clear quadratic effect with temperature ( $p \leq 0.02$ ) shown in Figure 4B, which  
372 is also represented by a significant coefficient for  $y^2$  ( $r^2 = 0.86$ ) in the corresponding

373 equation, indicating a decrease in chocolate aroma as the roasting conditions became  
374 more severe (and possibly over-cooked from a sensory perspective). However, the  
375 most sensory chocolate aroma was found in FJS flours. The sensory rankings of  
376 chocolate aroma (SCA) were 72 for FJS (40 min to 150 °C) compared to 70 for AJS (40  
377 min to 180°C) and the average of DJS was 60. Clearly, a fermentation or acidification  
378 process is necessary to produce chocolate aroma using jackfruit seeds, and it is  
379 possible to select the best roasting conditions for each treatment to optimize the  
380 sensory perception of chocolate aroma.

381 A range of descriptive terms were collected for the flours (Figure 5). All  
382 treatments were described with chocolate and coffee terms. In addition, sweet aroma  
383 attributes were used to described DJS flours (honey, milk, etc.) suggesting a relatively  
384 mild processing treatment. Unfermented cocoa is very bitter and astringent with little  
385 apparent chocolate flavor <sup>27,35</sup> whereas the unfermented jackfruit flour (from DJS) still  
386 had some chocolate aroma. For AJS flour, sweet aromas such as vanilla were similar to  
387 DJS flour, but other descriptors were used (e.g. earthy, rancid, acid, silage, fermented,  
388 green, etc.) which suggest that the chemical acidification process (rather than the  
389 natural fermentation process) may produce less desirable attributes which are not  
390 directly associate with food. However, FJS flour was described with fruity qualities  
391 (orange, passion fruit, cherry, jackfruit and guava). These aromas are likely to be  
392 related to fruity aldehydes, alcohols and esters which are products of the fermentation  
393 process. FJS flour was also described with caramel, soya, hazelnut and roast attributes  
394 suggesting a greater contribution from the Maillard reaction.

395 Overall, the sensory evaluation confirmed that a chocolate aroma can be  
396 generated from roasted jackfruit seeds, and demonstrated that it can be influenced by  
397 both the seed processing and the roasting conditions. The optimum chocolate aroma

398 score was obtained under moderate roasting conditions when the seeds had been  
399 fermented in a process similar to that used for fermenting cocoa beans, or acidified  
400 with acetic acid prior to roasting. However, the latter was described by the panel with  
401 additional less desirable terms. The best conditions were not necessarily obtained  
402 from the most severe roasting conditions and, for AFS flour, there was a very clear  
403 optimum, after which there was a decrease in chocolate aroma as the roasting  
404 conditions became more severe.

405 **Volatile aroma compounds in jackfruit seed flours.** Selection of aroma  
406 compounds was based on a survey of the literature (1997-2017), considering only  
407 those papers where the odor-active compounds in chocolate or other cocoa products  
408 had been established using GC-Olfactometry.<sup>7,8,25-27</sup> From each paper, the 15-20 most  
409 important aroma compounds for chocolate or cocoa aroma were identified and  
410 collated, based on either their flavor dilution factors (FD), odor activity values (OAV) or  
411 frequency of detection. The results of the survey are shown in Table S2. Chocolate  
412 aroma is a complex mixture of 30-50 odor-active compounds, none of which imparts a  
413 recognisable chocolate note. Some are present at very low concentrations (e.g. 2-  
414 acetyl-1-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone), often below the detection  
415 threshold when using SPME. Others, although contributory, are reminiscent of aromas  
416 very different to that of chocolate (e.g. 3-methylbutanoic acid, 2-methyl-3-  
417 methylthio)furan and 1-octen-3-one which impart cheesy, meaty and mushroom  
418 aromas respectively). In choosing just a few key compounds to monitor, our criteria  
419 were based on selecting those which had previously been identified as having high FD  
420 factors and high OAVs in chocolate or cocoa products, those which were relatively  
421 abundant, and those which had a relevant aroma. On this basis we selected 3-  
422 methylbutanal, one of the most abundant compounds and also one which at the

423 appropriate dilution can be described as cocoa and malty. Phenylacetaldehyde and 2-  
424 phenylethyl acetate were selected as compounds which contribute the floral character  
425 to chocolate. 2,3-Diethyl-5-methylpyrazine and trimethylpyrazine were selected as  
426 compounds which contribute the nutty earthy character. The approximate relative  
427 contributions of these, plus four other pyrazines, are shown in Table 5.

428 The most obvious difference is the fact that in the FJS flours, all selected  
429 volatiles, except 3-methylbutanal, were present at significantly higher concentrations  
430 compared to the respective AJS and DJS flours, particularly the pyrazines, and 2-  
431 phenylethyl acetate which was 50 times higher across all conditions. Since these  
432 compounds are amongst those which have been shown most frequently to be  
433 associated with chocolate aroma (Table S2), and have also been shown to be amongst  
434 the most odor-active, it is highly likely that these compounds are responsible for the  
435 high sensory scores for chocolate aroma in FJS.

436 Table 5 shows the significant differences within each pre-treatment (AJS, FJS or  
437 DJS). 2-Way ANOVA showed that for most compounds, under all treatments, there was  
438 a highly significant difference between flours prepared at different temperatures. In  
439 some cases, the roasting time was also significant, and the interaction between the  
440 two was significant in some cases.

441 It is interesting, however, that the key aroma compounds behaved quite  
442 differently with roasting time and temperature. With all three pre-treatments (AFS, FJS  
443 and DJS), 3-methylbutanal and phenylacetaldehyde showed a tendency to decrease as  
444 the more severe roasting conditions were employed. 3-Methylbutanal is both highly  
445 volatile and highly reactive: for example it readily undergoes aldol condensations with  
446 other aldehydes. Either or both of these may explain the decrease in concentration as  
447 the severity of the roasting process increased. This decrease in 3-methylbutanal may

448 also contribute to the decrease in chocolate aroma which was observed particularly in  
449 AJS and also in FJS as the roasting conditions became more extreme.

450 The trends for 2-phenylethyl acetate were not clear or consistent, and within  
451 each pre-treatment group, the differences due to different time-temperature  
452 combinations were small or non-significant.

453 2,3-Diethyl-5-methylpyrazine, the most odor-active of the pyrazines identified in  
454 most chocolate and cocoa products, showed a tendency to increase with increasing  
455 severity of the roasting conditions, as is often the case for pyrazines. However, for  
456 trimethylpyrazine, another important compound in chocolate aroma, the trends were  
457 less clear, and in FJS it (and tetramethylpyrazine) tended to decrease with more severe  
458 conditions, although both tended to increase slightly in AJS and DJS. The  
459 dimethylpyrazines also tended to increase with increased roasting conditions in AJS  
460 and DJS, but did not vary much in FJS.

461 In AJS and DJS, as the roasting conditions became more severe, the 3-  
462 methylbutanal decreased whereas the 2,3-diethyl-5-methylpyrazine increased. Both  
463 being important for chocolate aroma, this is consistent with the sensory data which  
464 showed an optimum sensory chocolate aroma under moderate roasting conditions for  
465 AJS and DJS. In addition, the more severe conditions might also promote the formation  
466 of other pyrazines which at higher concentration would impart more roasted and  
467 burnt notes, as described in some DJS and FJS samples.

468 In FJS, most of the compounds were not sensitive to changes in roasting  
469 conditions, although 3-methylbutanal, phenylacetaldehyde and trimethylpyrazine  
470 tended to decrease. This is consistent with the sensory perception of chocolate aroma  
471 in FJS which showed a tendency to decrease as the roasting temperature increased.

472           **Response surface methodology.** The response surfaces for 2,3-diethyl-5-  
473 methylpyrazine are shown in Figures 6 A-C and the corresponding equations in Table  
474 S1. The most noticeable difference between the treatments is the relative  
475 concentration of 2,3-diethyl-5-methylpyrazine in FJS flour which was approximately  
476 five and three times bigger than in flour from DJS and AJS respectively. Figure 6 clearly  
477 demonstrates the positive influence of time and temperature on the formation of this  
478 compound. However, in AJS and FJS flour, none of the coefficients relating to roast  
479 time or temperature had a significant impact on the response at  $p < 0.05$ , either linear  
480 or quadratic, although they were significant at  $p < 0.1$ . P-values were 0.07 and 0.09  
481 respectively and positive, confirming the positive effect of temperature.

482           Direct comparison of the formation of 2,3-diethyl-5-methylpyrazine at the lowest  
483 and highest temperature ( $t = 40$  min in all cases) showed that it was significantly higher  
484 in all three flours when the higher temperature was employed (Table 5). Furthermore,  
485 in DJS, four out of the six pyrazines monitored also showed a significant increase (all at  
486  $p < 0.001$ ) and in AJS five out of six showed a significant increase (four of these at  
487  $p < 0.001$ ). This is in agreement with many other studies<sup>10,37</sup> that show that pyrazine  
488 formation in general is greatly influenced by temperature. Queiroz and Garcia<sup>32</sup>  
489 evaluated roasting time and temperature for cupuaçu seeds and concluded that  
490 increased time resulted in greater pyrazine formation and increased the scores for  
491 chocolate in the sensory profile. For cupuaçu, the best roasting conditions were 150°C  
492 for 42 min. For cocoa beans, Farah et al.<sup>10</sup> reported an increase in the concentration of  
493 pyrazines, particularly tetramethylpyrazine, when they roasted beans at temperatures  
494 close to 160°C.

495           Figure 6 (A, B and C) shows that the greatest relative concentration of 2,3-  
496 dimethyl-5-methylpyrazine was formed in dry, acidified and fermented flour when we

497 used 171 or 186, 201 and 180°C, respectively. These temperatures are higher than  
498 those milder conditions (110 - 140 °C for 20 - 50 min) reported for cocoa by Jinap et  
499 al.<sup>36</sup> or Afoakwa et al.<sup>13</sup> (120-150 °C for 5-120 min).

500 The response surfaces for 3-methylbutanal are shown in Figures 6 D-F. They  
501 clearly demonstrate that, contrary to 2,3-diethyl-5-methylpyrazine, high time and  
502 temperature are not the most favorable roasting conditions for the formation of 3-  
503 methylbutanal. The equation in Table S1 shows that the linear temperature coefficient  
504 in AJS is significant ( $p=0.03$ ) and negative, indicating that the lower temperatures  
505 produce a greater response. For AJS and FJS, the lowest temperatures generated the  
506 most 3-methylbutanal, but in DJS, there was an optimum around the mid-point,  
507 consistent with the data presented in Table 5. Optimum temperatures for 3-  
508 methylbutanal in DJS, AJS and FJS were 171, 165 and 154 °C, respectively, closer to  
509 those used for cocoa roasting.

510 The similarity of the optimum jackfruit roasting conditions, compared to cocoa,  
511 may be due to the fact that jackfruit seeds have a similar composition compared to  
512 cocoa beans, although dried jackfruit seeds have a lower lipid content (0.4% compared  
513 to dried cocoa beans which have range between 53 and 39%).<sup>12,13</sup>

514 Whilst we have selected a few compounds as a marker of chocolate flavor, it is  
515 clear from these results that there are other factors involved, particularly those  
516 associated with the fermented product. Further work is currently being carried out to  
517 investigate more thoroughly the contribution from a wider range of volatile  
518 compounds.

519 Waste jackfruit seeds have been roasted to prepare a flour which has a chocolate  
520 aroma. Moisture, pH and color were similar to those of cocoa, and different aroma  
521 profiles were obtained by acidifying or fermenting the seeds prior to roasting under



522 different time/temperature combinations. Optimum chocolate aroma scores were  
523 achieved when either fermentation or acidification was performed prior to roasting,  
524 and fermentation produced fewer off-notes. Utilization of this local waste stream can  
525 provide a new revenue stream for local farmers and boost local economies.

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#### 530 ASSOCIATED CONTENT

531 Table S1: Equations, coefficients,  $r^2$  and p-value for all equations derived from  
532 the response surface methodology. Table S2 Summary of odor-active compounds  
533 found in chocolate and cocoa 1997-2017. This material is available free of charge via  
534 the Internet at <http://pubs.acs.org>.

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643

644 FIGURE CAPTIONS

645 Figure 1A. Summary of jackfruit seed processing. DJS, AJS and FJS are dried, acidified  
646 and fermented jackfruit seeds respectively.

647 Figure 1B. Central composite design using two factors each at 5 levels; DJS, AJS and FJS  
648 are dried, acidified and fermented jackfruit seeds respectively.

649 Figure 2. Experimental design used for sensory ranking test where T= number of  
650 samples; k= number of samples in each ranking test; r= number of times each sample  
651 was shown within each block; B= number of panelists in each block; L= number of  
652 times the samples were shown together; E = dependability of the analysis; Z= number  
653 times the block was repeated.<sup>25</sup>

654 Figure 3. Variables followed during the processing of the seeds prior to roasting: A = pH  
655 during fermentation process; B = pH during acidification process; C = total titratable  
656 acidity (g/100g) during fermentation process and D = temperature (°C) during  
657 fermentation process; close = anaerobic 6-7 days; open = aerobic 7-8 days.

658 Figure 4. Response surfaces for roasted jackfruit seeds. A, B and C = total sensory  
659 chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS respectively.

660 Figure 5. Representation of aroma attributes used freely by the panelists to describe  
661 the roasted flours from fermented, dried and acidified jackfruit seed

662 Figure 6. Response surfaces for roasted jackfruit seeds. A, B and C = 2,3-diethyl-5-  
663 methylpazine for flour from DJS, AJS and FJS respectively. D, E and F = 3-  
664 methylbutanal from DJS, AJS and FJS respectively.

Table 1. Results from preliminary ranking experiment using different pre-exposure conditions of the roasted flours prior to ranking

coded values		roasting conditions		total ranking score
x	y	actual values	actual values	for sensory chocolate
		time (s)	temperature (°C)	aroma <sup>a</sup>
1	-1	120	25	13 a
-1	-1	30	25	15 a
-1	1	30	48	16 a
1	1	120	48	20 a
0	0	60	36.5	22 a
0	0	60	36.5	21 a
0	0	60	36.5	19 a

T=7; k=3; r=3; b=21; L=1; E=78 where T = number of samples; k = number of samples in each ranking test; r = number of times each sample was shown within each block; B= number of panelists in each block; L= number of times the samples were shown together; E = dependability of the analysis; Z= number times the block was repeated<sup>24</sup>.

<sup>a</sup>values with the same letter are not significantly different at p<0.05

Table 2. Mean  $\pm$  standard error (n=3) pH, water activity, moisture L\* and chroma\* of the roasted jackfruit seed flours showing mean values.

x (time)	y (temp)	pH	a <sub>w</sub>	moisture %	L*	chroma
Flour from dried jackfruit seeds (DJS)						
0	1.41	5.4 $\pm$ 0.04 <sup>a</sup>	0.33 $\pm$ 0.02 <sup>d</sup>	1.0 $\pm$ 0.3 <sup>d</sup>	53 $\pm$ 3 <sup>e</sup>	33.6 $\pm$ 0.2 <sup>a</sup>
1	1	5.3 $\pm$ 0.01 <sup>d</sup>	0.32 $\pm$ 0.02 <sup>d</sup>	1.2 $\pm$ 0.4 <sup>d</sup>	55 $\pm$ 1 <sup>de</sup>	33.2 $\pm$ 0.2 <sup>ab</sup>
-1	1	5.3 $\pm$ 0.02 <sup>bcd</sup>	0.32 $\pm$ 0.01 <sup>d</sup>	1.3 $\pm$ 0.3 <sup>d</sup>	58 $\pm$ 3 <sup>cde</sup>	32.1 $\pm$ 0.6 <sup>bc</sup>
-1.41	0	5.3 $\pm$ 0.02 <sup>d</sup>	0.37 $\pm$ 0.01 <sup>c</sup>	3.7 $\pm$ 0.8 <sup>b</sup>	59 $\pm$ 3 <sup>bcd</sup>	32.1 $\pm$ 0.2 <sup>bc</sup>
1.41	0	5.4 $\pm$ 0.02 <sup>b</sup>	0.31 $\pm$ 0.01 <sup>d</sup>	2.0 $\pm$ 0.2 <sup>cd</sup>	59 $\pm$ 0.4 <sup>bcd</sup>	33.3 $\pm$ 0.6 <sup>ab</sup>
0	0	5.2 $\pm$ 0.01 <sup>e</sup>	0.38 $\pm$ 0.01 <sup>c</sup>	3.4 $\pm$ 0.6 <sup>bc</sup>	60 $\pm$ 2 <sup>abcde</sup>	32.9 $\pm$ 0.4 <sup>ab</sup>
0	0	5.3 $\pm$ 0.01 <sup>d</sup>	0.42 $\pm$ 0.03 <sup>abc</sup>	2.9 $\pm$ 0.5 <sup>bc</sup>	60 $\pm$ 3 <sup>abcd</sup>	33.2 $\pm$ 0.3 <sup>ab</sup>
0	0	5.2 $\pm$ 0.01 <sup>e</sup>	0.43 $\pm$ 0.01 <sup>a</sup>	3.2 $\pm$ 0.3 <sup>bc</sup>	60 $\pm$ 2 <sup>abcde</sup>	32.5 $\pm$ 0.5 <sup>abc</sup>
1	-1	5.2 $\pm$ 0.01 <sup>e</sup>	0.44 $\pm$ 0.01 <sup>a</sup>	5.8 $\pm$ 0.9 <sup>a</sup>	64 $\pm$ 2 <sup>abc</sup>	31.6 $\pm$ 0.5 <sup>c</sup>
-1	-1	5.4 $\pm$ 0.00 <sup>b</sup>	0.40 $\pm$ 0.02 <sup>abc</sup>	5.8 $\pm$ 0.8 <sup>a</sup>	66.0 $\pm$ 1.6 <sup>a</sup>	31.3 $\pm$ 0.6 <sup>c</sup>
0	-1.41	5.3 $\pm$ 0.01 <sup>bc</sup>	0.40 $\pm$ 0.01 <sup>bc</sup>	6.4 $\pm$ 0.2 <sup>a</sup>	65.1 $\pm$ 1.5 <sup>ab</sup>	31.2 $\pm$ 0.5 <sup>c</sup>
Flour from acidified jackfruit seeds (AJS)						
0	1.41	5.6 $\pm$ 0.01 <sup>a</sup>	0.5 $\pm$ 0.01 <sup>abc</sup>	0.5 $\pm$ 0.4 <sup>d</sup>	61.7 $\pm$ 0.9 <sup>cd</sup>	32.0 $\pm$ 0.8 <sup>a</sup>
1	1	5.6 $\pm$ 0.01 <sup>a</sup>	0.50 $\pm$ 0.02 <sup>a</sup>	0.9 $\pm$ 0.9 <sup>cd</sup>	60.5 $\pm$ 1.6 <sup>d</sup>	31.5 $\pm$ 0.8 <sup>a</sup>
-1	1	5.2 $\pm$ 0.01 <sup>f</sup>	0.47 $\pm$ 0.01 <sup>c</sup>	1.6 $\pm$ 0.2 <sup>bcd</sup>	65.3 $\pm$ 0.9 <sup>abcd</sup>	30.2 $\pm$ 0.2 <sup>ab</sup>
1.41	0	5.3 $\pm$ 0.01 <sup>c</sup>	0.47 $\pm$ 0.01 <sup>bc</sup>	1.9 $\pm$ 0.7 <sup>abcd</sup>	66.1 $\pm$ 0.8 <sup>abc</sup>	31.1 $\pm$ 0.2 <sup>a</sup>
1.41	0	5.2 $\pm$ 0.01 <sup>de</sup>	0.48 $\pm$ 0.01 <sup>abc</sup>	2.4 $\pm$ 0.6 <sup>abc</sup>	64.7 $\pm$ 2.2 <sup>abcd</sup>	30.5 $\pm$ 0.7 <sup>ab</sup>
0	0	5.3 $\pm$ 0.01 <sup>cd</sup>	0.50 $\pm$ 0.01 <sup>ab</sup>	1.9 $\pm$ 0.7 <sup>bcd</sup>	66.1 $\pm$ 1.4 <sup>abc</sup>	30.2 $\pm$ 0.5 <sup>ab</sup>
0	0	5.2 $\pm$ 0.01 <sup>ef</sup>	0.47 $\pm$ 0.01 <sup>bc</sup>	2.0 $\pm$ 0.5 <sup>bcd</sup>	65.3 $\pm$ 0.4 <sup>abcd</sup>	30.8 $\pm$ 0.5 <sup>ab</sup>
0	0	4.9 $\pm$ 0.02 <sup>h</sup>	0.47 $\pm$ 0.01 <sup>bc</sup>	1.4 $\pm$ 0.7 <sup>bcd</sup>	64.3 $\pm$ 3.0 <sup>abcd</sup>	31.1 $\pm$ 1.0 <sup>a</sup>
1	-1	5.0 $\pm$ 0.02 <sup>g</sup>	0.46 $\pm$ 0.01 <sup>c</sup>	2.6 $\pm$ 0.8 <sup>ab</sup>	63.2 $\pm$ 2.9 <sup>bcd</sup>	31.2 $\pm$ 0.8 <sup>a</sup>
-1	-1	5.2 $\pm$ 0.03 <sup>ef</sup>	0.48 $\pm$ 0.01 <sup>abc</sup>	2.9 $\pm$ 0.2 <sup>ab</sup>	67.3 $\pm$ 1.9 <sup>ab</sup>	30.6 $\pm$ 0.8 <sup>ab</sup>
0	-1.41	5.3 $\pm$ 0.01 <sup>b</sup>	0.48 $\pm$ 0.01 <sup>abc</sup>	3.8 $\pm$ 0.2 <sup>a</sup>	69.3 $\pm$ 0.8 <sup>a</sup>	29.1 $\pm$ 0.2 <sup>b</sup>
Flour from fermented jackfruit seeds (FJS)						
0	1.41	5.1 $\pm$ 0.00 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>e</sup>	2.3 $\pm$ 0.5 <sup>f</sup>	49.4 $\pm$ 1.9 <sup>ab</sup>	27.6 $\pm$ 0.5 <sup>ab</sup>
1	1	5.0 $\pm$ 0.01 <sup>b</sup>	0.42 $\pm$ 0.01 <sup>ab</sup>	2.3 $\pm$ 0.1 <sup>f</sup>	50.1 $\pm$ 1.8 <sup>ab</sup>	27.8 $\pm$ 0.4 <sup>ab</sup>
-1	1	4.8 $\pm$ 0.01 <sup>c</sup>	0.39 $\pm$ 0.01 <sup>cd</sup>	2.5 $\pm$ 0.2 <sup>f</sup>	53.5 $\pm$ 1.3 <sup>a</sup>	28.1 $\pm$ 0.3 <sup>ab</sup>
-1.41	0	4.7 $\pm$ 0.01 <sup>g</sup>	0.38 $\pm$ 0.01 <sup>d</sup>	4.1 $\pm$ 0.5 <sup>bcd</sup>	52.3 $\pm$ 3.5 <sup>a</sup>	28.9 $\pm$ 0.7 <sup>a</sup>
1.41	0	4.8 $\pm$ 0.01 <sup>d</sup>	0.43 $\pm$ 0.01 <sup>a</sup>	3.6 $\pm$ 0.1 <sup>cde</sup>	53.7 $\pm$ 0.6 <sup>a</sup>	28.8 $\pm$ 0.4 <sup>a</sup>
0	0	4.8 $\pm$ 0.02 <sup>ef</sup>	0.39 $\pm$ 0.01 <sup>cd</sup>	3.3 $\pm$ 0.3 <sup>def</sup>	52.8 $\pm$ 1.9 <sup>a</sup>	27.7 $\pm$ 0.5 <sup>ab</sup>
0	0	4.7 $\pm$ 0.00 <sup>f</sup>	0.40 $\pm$ 0.01 <sup>bc</sup>	3.0 $\pm$ 0.4 <sup>ef</sup>	44.5 $\pm$ 3.4 <sup>b</sup>	26.8 $\pm$ 0.8 <sup>b</sup>
0	0	4.8 $\pm$ 0.01 <sup>d</sup>	0.37 $\pm$ 0.01 <sup>d</sup>	3.9 $\pm$ 0.5 <sup>cde</sup>	49.5 $\pm$ 2.1 <sup>ab</sup>	28.4 $\pm$ 0.4 <sup>a</sup>
1	-1	4.7 $\pm$ 0.01 <sup>g</sup>	0.41 $\pm$ 0.01 <sup>ab</sup>	4.5 $\pm$ 0.1 <sup>bc</sup>	51.5 $\pm$ 1.1 <sup>a</sup>	27.7 $\pm$ 0.3 <sup>ab</sup>
-1	-1	4.5 $\pm$ 0.02 <sup>h</sup>	0.41 $\pm$ 0.01 <sup>ab</sup>	5.7 $\pm$ 0.2 <sup>a</sup>	51.3 $\pm$ 2.9 <sup>a</sup>	27.9 $\pm$ 0.4 <sup>ab</sup>
0	-1.41	4.8 $\pm$ 0.01 <sup>de</sup>	0.38 $\pm$ 0.01 <sup>cd</sup>	5.1 $\pm$ 0.4 <sup>ab</sup>	49.0 $\pm$ 2.9 <sup>ab</sup>	27.9 $\pm$ 0.4 <sup>ab</sup>

Within each column for each treatment, values with the same letter are not significantly different from each other ( $p \leq 0.05$ ) using the Tukey test.

Table 3. Proximate composition (%  $\pm$  standard error) of jackfruit flours roasted under the best conditions.

flour <sup>a</sup>	moisture	lipids	proteins	ash	fiber	
					insoluble	soluble
DJS	5.88 $\pm$ 0.9 a <sup>b</sup>	0.40 $\pm$ 0.05 a	11.20 $\pm$ 0.7 b	2.90 $\pm$ 0.02 b	10.34 $\pm$ 0.05 b	3.88 $\pm$ 0.010 a
AJS	1.38 $\pm$ 0.7 b	0.30 $\pm$ 0.05 b	11.16 $\pm$ 0.5 b	2.44 $\pm$ 0.12 c	9.29 $\pm$ 0.01 c	2.68 $\pm$ 0.003 b
FJS	5.10 $\pm$ 0.4 a	0.50 $\pm$ 0.03 a	14.82 $\pm$ 0.5 a	4.70 $\pm$ 0.09 a	18.9 $\pm$ 0.8 a	3.34 $\pm$ 0.002 a

<sup>a</sup>DJS = dried jackfruit seeds (47min at 171 °C); AJS = acidified jackfruit seeds (40 min at 180 °C); FJS =fermented jackfruit seeds (40 min at 150 °C)

<sup>b</sup>Mean (n=3), within each column, values with the same letter are not significantly different from each other ( $p \leq 0.05$ ) using the Tukey test.



Table 4. Total sensory chocolate aroma (SCA) ranking score for flour from DJS, AJS and FJS.

	coded values		total ranking scores for sensory chocolate aroma <sup>a</sup>		
	x (time)	y (temp)	DJS	AJS	FJS
incomplete block					
1	1	-1	66 a <sup>b</sup>	67 ab	68 ab
2	-1	-1	52 a	58 ab	66 ab
3	-1	1	58 a	63 ab	60 ab
4	1	1	66 a	51 ab	51 bc
5	0	1.41	51 a	42 c	44 c
6	0	-1.41	57 a	54 bc	72 a
7	1.41	0	62 a	69 a	61 ab
8	-1.41	0	56 a	66 ab	55 bc
blend	0	0	62 a	70 a	63 ab
complete block <sup>c</sup>					
9	0	0	64 k	68 k	71 k
10	0	0	62 k	68 k	65 k
11	0	0	60 k	74 k	53 k

<sup>a</sup>DJS = dried jackfruit seeds; AJS = acidified jackfruit seeds; FJS - fermented jackfruit seeds.

<sup>b</sup>Within each column, means followed by the same letters are not significantly different ( $p \leq 0.05$ ).

<sup>c</sup>Values are transformed for comparison with incomplete block.

Table 5. Approximate relative concentrations of selected volatiles in roasted jackfruit seed flours

LRI <sup>a</sup>	compound ID <sup>b</sup>	roasting conditions											significance <sup>c</sup>			
		roasting temp	roasting time	150 °C	156 °C	156 °C	171 °C	171 °C	171 °C	171 °C	171 °C	186 °C	186 °C	192 °C	T	t
<b>DRIED JACKFRUIT (DJS)</b>																
		roasting temp	150 °C	156 °C	156 °C	171 °C	171 °C	171 °C	171 °C	171 °C	186 °C	186 °C	192 °C			
		roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	6.0 <sup>d</sup>	7.1 bcd	8.1 abc	8.8 ab	8.1 abc	9.7 a	6.5 cd	5.4 d	7.2 bcd	5.6 d	3.4 e	***	*	ns	
827	2-methylpyrazine	4.3 fg	3.3 g	4.6 fg	6.6 efg	15 d	12 de	11 def	124 a	25 c	33 b	32 b	***	***	ns	
916	2,5/6-dimethylpyrazine	39 e	54 e	66 de	101 cd	121 bc	124 bc	96 cd	123 bc	167 a	185 a	155 ab	***	ns	ns	
922	2,3-dimethylpyrazine	12 g	11 g	72 b	19 fg	30 def	34 d	21 efg	33 de	49 c	63 b	110 a	***	***	***	
1008	2,3,5-trimethylpyrazine	25 abc	23 abc	11 c	19 bc	25 abc	26 abc	19 bc	44 ab	42 a	36 ab	35 ab	*	ns	ns	
1058	phenylacetaldehyde	11.2a	8.9ab	9.7a	8.1ab	6.6abc	7.8ab	10.6a	6.8abc	6.8abc	1.9c	3.4bc	***	ns	ns	
1091	2,3,5,6-tetramethylpyrazine	110 cde	92 cde	68 e	69 e	88 e	139 bcd	110 cde	91 de	180 ab	210 a	140 bc	***	ns	ns	
1157	2,3-diethyl-5-methylpyrazine	1.5 fg	1.3 g	1.9 ef	1.9 ef	2.6 cd	3.2 b	2.2 de	4.3 a	3.9 a	4.3 a	3 bc	***	***	ns	
1263	2-phenylethyl acetate	0.1 ab	0.1 ab	0.08 bc	0.09 bc	0.08 c	0.07 c	0.08 bc	0.12 a	0.13 a	0.12 a	0.08 c	**	***	ns	
<b>ACIDIFIED JACKFRUIT (AJS)</b>																
		roasting temp	159 °C	165 °C	165 °C	180 °C	180 °C	180 °C	180 °C	180 °C	195 °C	195 °C	201 °C			

	roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	11.2 bc	13.2 a	12.2 ab	10.3 c	10.1 c	4.4 ef	7.5 d	7.8 d	5.2 e	4 f	2.3 g	***	*	ns
827	2-methylpyrazine	2.7 d	3.5 d	11.6 c	1.2 d	1.6 d	28 a	21 b	11 c	2.6 d	25 ab	29 a	**	**	ns
916	2,5/6-dimethylpyrazine	66 d	74 d	110 c	110 c	120 bc	146 a	137 ab	86 d	113 c	140 ab	123 bc	***	***	ns
922	2,3-dimethylpyrazine	37 g	126 a	58 f	59 f	68 ef	97 c	79 d	8.7 h	73 de	110 b	110 bc	***	***	***
1008	2,3,5-trimethylpyrazine	52 abc	65 a	28 cd	30 bcd	35 abcd	44 abc	43 abc	18 d	45 abc	59 abc	65 ab	ns	*	*
1058	phenylacetaldehyde	10.7bc	11.8ab	10.8b	9.7bcd	7.4cd	14.8a	7.1d	3.3e	3.4e	2.7e	0.9e	ns	**	ns
1091	2,3,5,6-tetramethylpyrazine	28 cd	67 bc	38 bcd	44 bcd	59 bcd	57 bcd	79 bc	12 d	88 b	160 a	150 a	***	ns	**
1157	2,3-diethyl-5-methylpyrazine	1.2 f	1.1 f	1.8 ef	2.1 de	2.8 d	4.6 c	4.2 c	1.8 ef	4.9 bc	7.8 a	5.4 b	***	***	**
1263	2-phenylethyl acetate	0.13 d	0.14 d	0.16cd	0.14 d	0.17bcd	0.15d	0.19abc	0.07e	0.20ab	0.21a	0.19abc	***	***	ns

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FERMENTED JACKFRUIT SEEDS (FJS)

	roasting temp	150 °C	154 °C	154 °C	165 °C	165 °C	165 °C	165 °C	165 °C	176 °C	176 °C	180 °C			
	roasting time	40 min	35 min	45 min	33 min	40 min	40 min	40 min	47 min	35 min	45 min	40 min			
657	3-methylbutanal	7 abcde	11 a	9.8 ab	9 abc	8.4 abcd	5.1 def	6.8 bcde	4.3 ef	5.9 cdef	2.3 f	2.8 f	***	*	ns
827	2-methylpyrazine	100 abc	86 cd	123 ab	86 bcd	100 abc	53 d	130 a	129 a	123 a	113 abc	110 abc	ns	ns	ns
916	2,5/6-dimethylpyrazine	375 ab	280 bc	450 a	250 bc	290 bc	234 c	312 bc	266 bc	278 bc	215 c	240 c	*	ns	*
922	2,3-dimethylpyrazine	487 ab	410 bc	600 a	420 bc	400 bc	421 bc	513 ab	510 ab	511 ab	402 bc	320 c	*	ns	**
1008	2,3,5-trimethylpyrazine	560 abc	480 bc	690 a	380 bcd	370 cd	110 e	130 e	98 e	210 de	130 e	270 de	***	ns	ns

1058	phenylacetaldehyde	20b	27a	20b	15bc	11cde	10cde	13c	12cd	10cde	6.9de	6.0e	***	ns	ns
1091	2,3,5,6-tetramethyl-pyrazine	4330 ab	3840 bc	5300 a	4380 ab	4070 b	3840 bc	5320 a	4190 b	4200 b	2700 d	2830 cd	**	ns	**
1157	2,3-diethyl-5-methyl-pyrazine	7.5 f	11 ef	14 bcd	12 de	16 abc	13 cde	18 a	16 ab	16 ab	19 a	15abc	***	*	ns
1263	2-phenylethyl acetate	7.3 c	12 a	11 ab	7.6 c	7 cd	6.6 cd	9.5 b	10.1 b	7.6 c	6.3 cd	5.5 b	***	*	ns

<sup>a</sup> Linear retention index on RTX5MS column (30m), calculated from a linear equation between each pair of straight chain alkanes C<sub>6</sub>–C<sub>30</sub>.

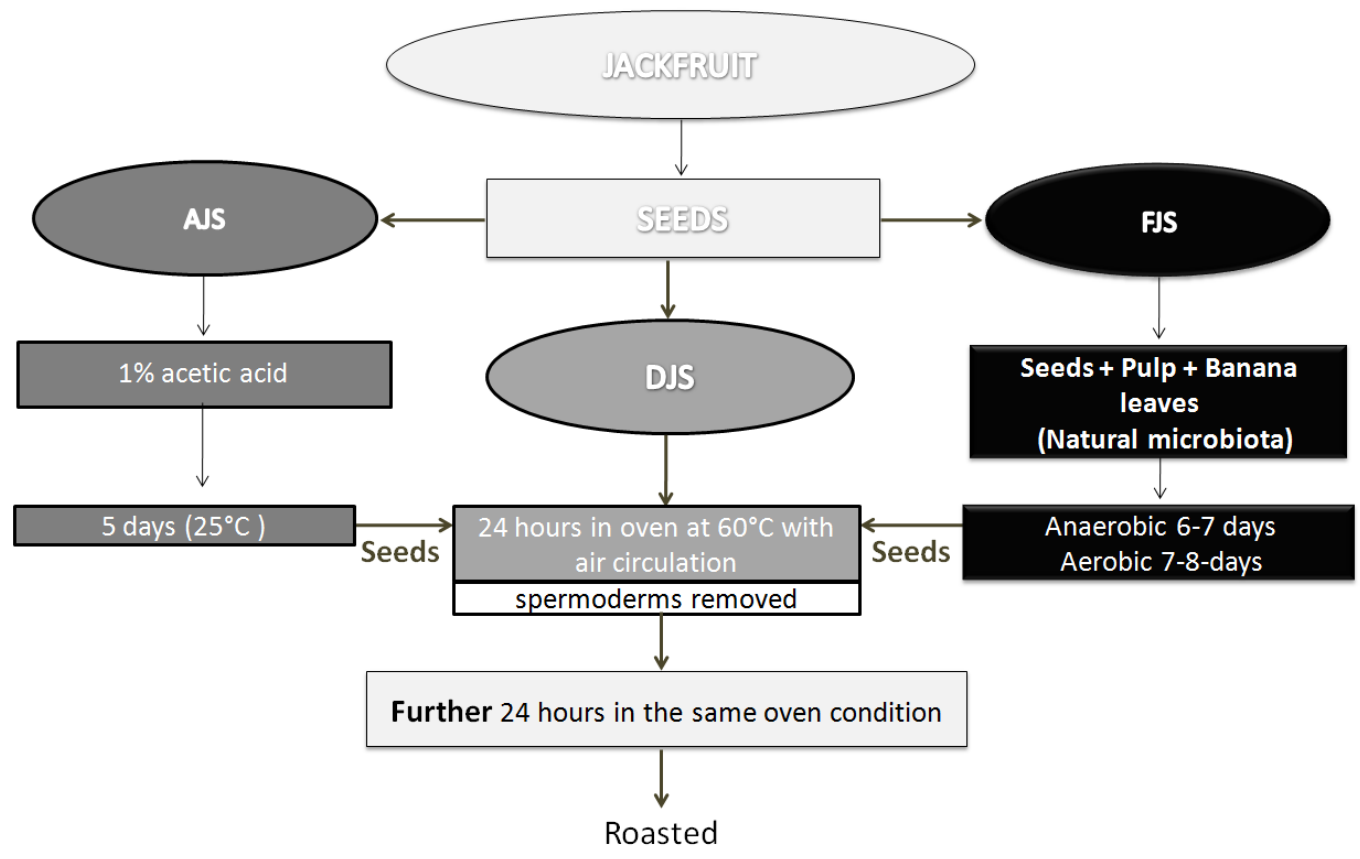
<sup>b</sup> Identity: identity of all compounds confirmed by comparison of mass spectrum and LRI with that of the authentic standard run under similar conditions.

<sup>c</sup>S: Significance of differences between samples within one pre-treatment (AJS, FJS or DJS) - probability, obtained from 2-way ANOVA, that there is a difference between means; ns = no significant difference between means (p>0.05); \* significant at the 5% level; \*\* significant at the 1% level; \*\*\* significant at the 0.1% level,

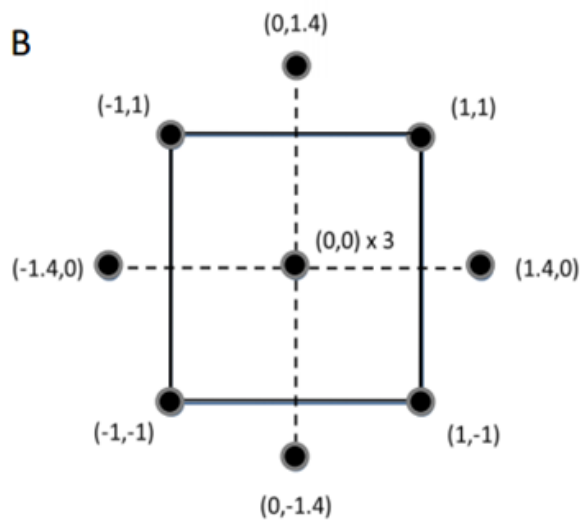
with respect to; T = roasting temperature, t = roasting time, T×t interaction between roasting time and temperature.

<sup>d</sup> Mean (n=3) relative concentration (μg/kg) = peak area of compound × concentration of internal standard (ISTD) / peak area of ISTD, nd = not detected. Within each row, cells containing the same letter are not significantly different from each other at p<0.05.

A



B



Factors	Levels	Levels				
		-1.41	-1	0	1	1.41
Time (min), x		33	35	40	45	47
	DJS	150	156	171	186	192
Temperature (°C), y	AJS	159	165	180	195	201
	FJS	150	154	165	176	180

Figure 1A.

Figure 1B.

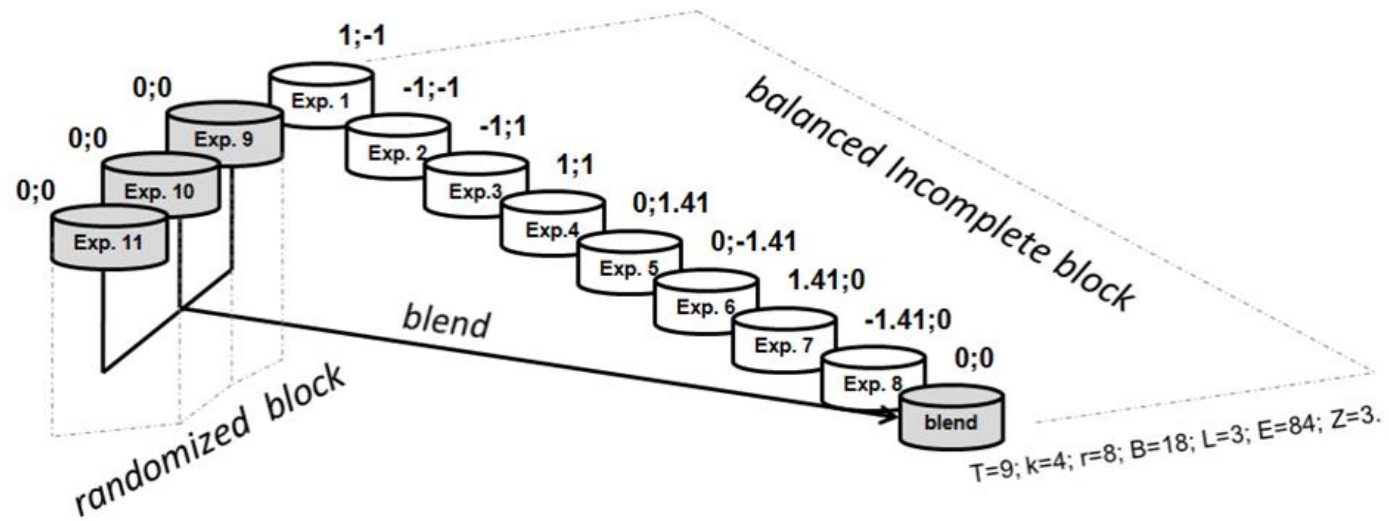


Figure 2

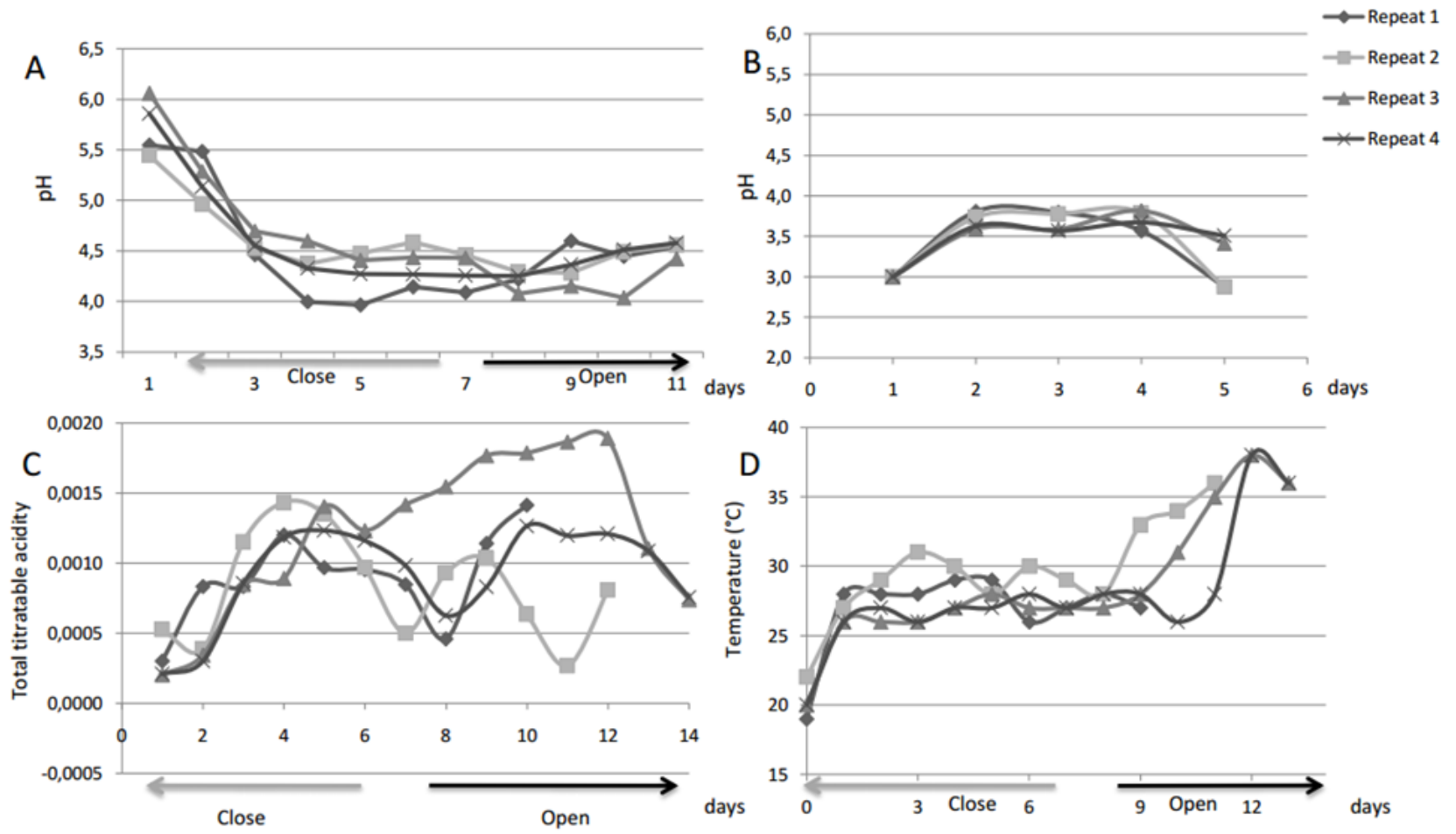
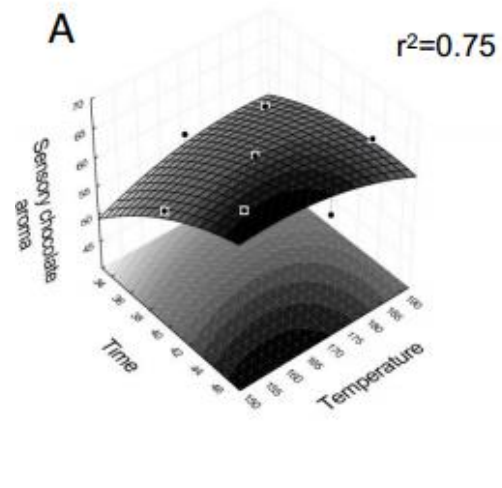
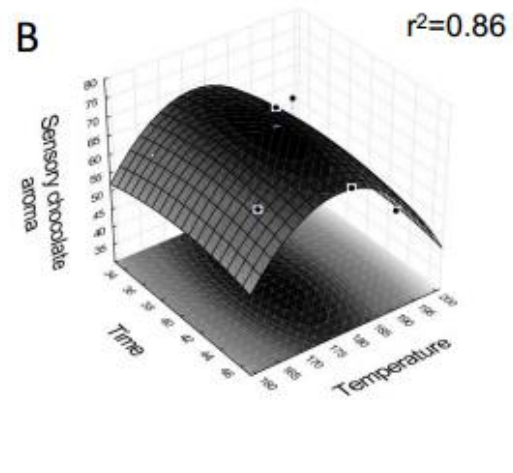


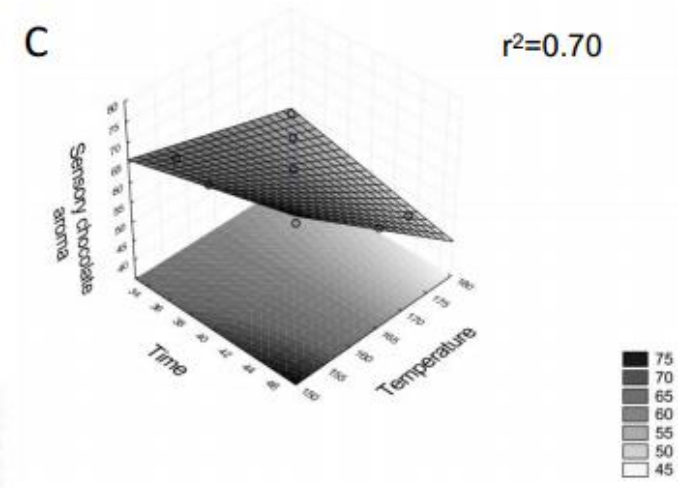
Figure 3



$$SCA_{DJS} = -324.84 + 7.807x + 2.514y - 0.0452x^2 - 0.005y^2 - 0.02xy$$



$$SCA_{AJS} = -2017.58 + 15.835x + 19.9425y - 0.0628839x^2 - 0.04942y^2 - 0.06xy$$



$$SCA_{FJS} = -621.7 + 13.46x + 5.761y - 0.0608x^2 - 0.0134y^2 - 0.05188xy$$

Figure 4



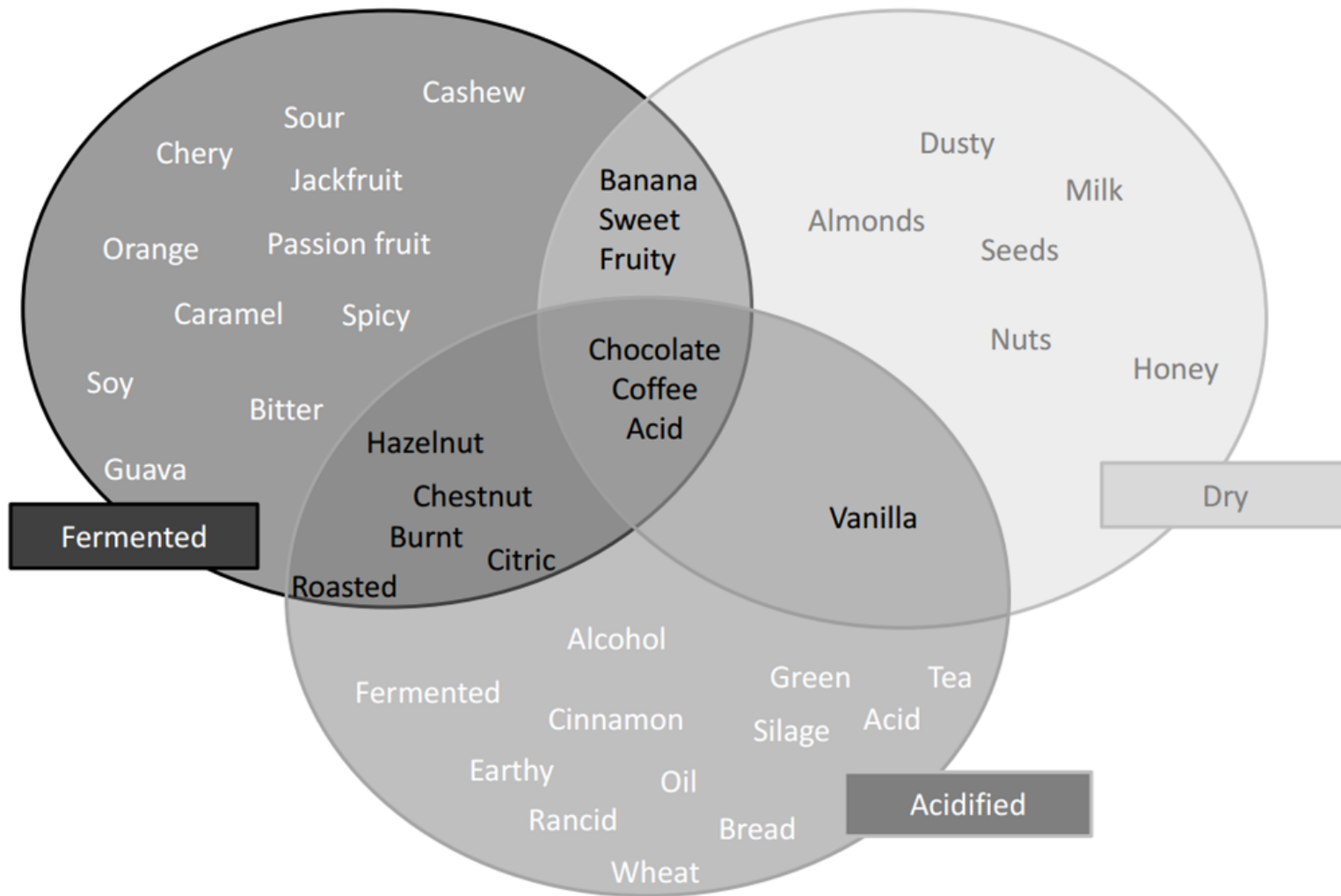


Figure 5

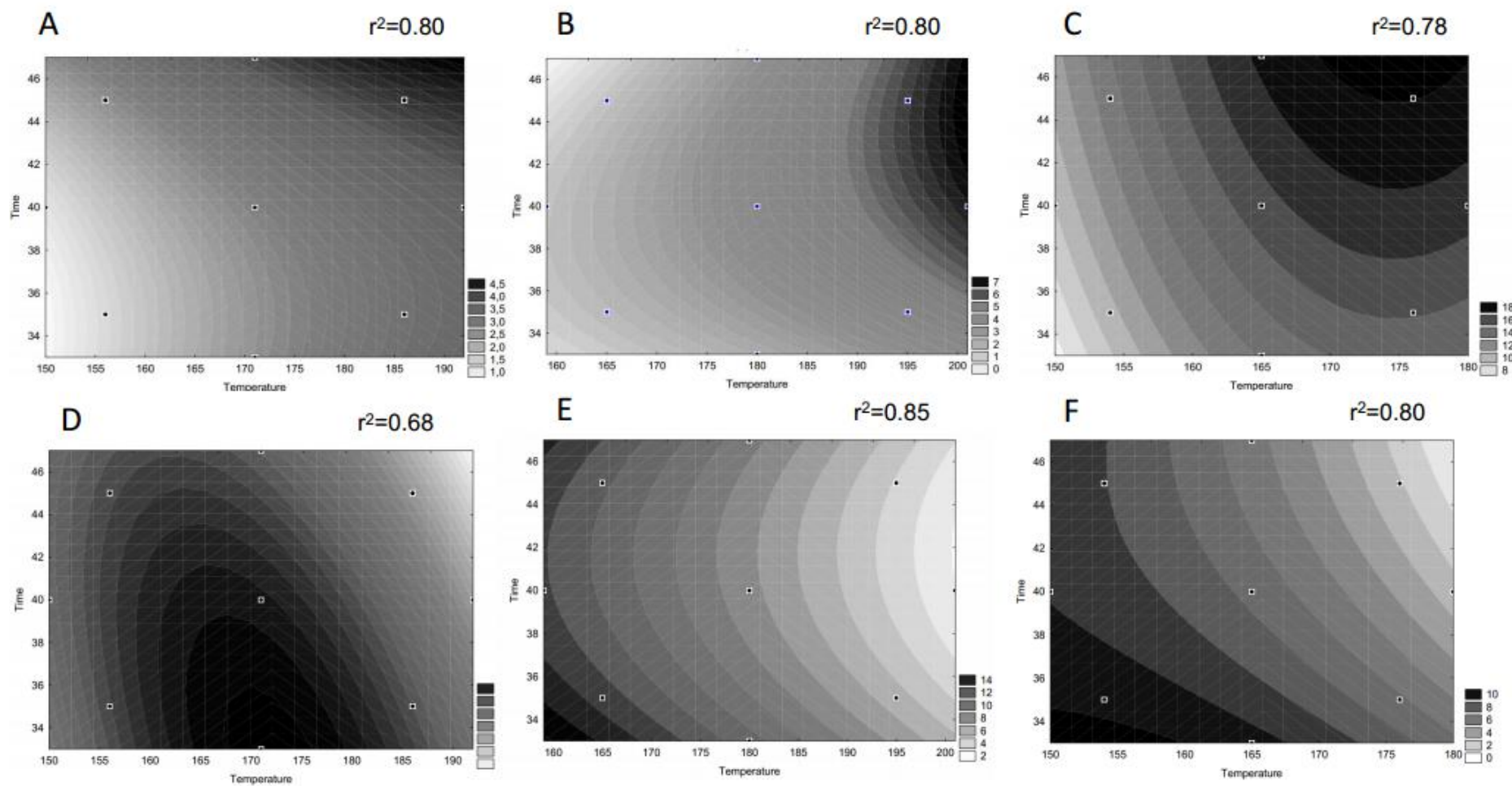


Figure 6

# TOC GRAPHIC

