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▶ To cite this version:

Hervé Colinet, David Renault. Dietary live yeast alters metabolic profiles, protein biosynthesis and thermal stress tolerance of Drosophila melanogaster. Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology, Elsevier, 2014, 170, pp.6-14. <10.1016/j.cbpa.2014.01.004>. <hal-00999532>

HAL Id: hal-00999532

https://hal-univ-rennes1.archives-ouvertes.fr/hal-00999532

Submitted on 3 Jun 2014

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Dietary live yeast alters metabolic profiles, protein biosynthesis and thermal stress tolerance of *Drosophila melanogaster*

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Running headline: nutrition affects cold tolerance

Keywords: fruit fly, nutrition, live yeast, metabolic fingerprinting, cold stress

23 ***Abstract***

24

25 The impact of nutritional factors on insect's life-history traits such as reproduction and
26 lifespan has been excessively examined; however, nutritional determinant of insect's thermal
27 tolerance has not received a lot of attention. Dietary live yeast represents a prominent source
28 of proteins and amino acids for laboratory-reared drosophilids. In this study, *Drosophila*
29 *melanogaster* adults were fed on diets supplemented or not with live yeast. We hypothesized
30 that manipulating nutritional conditions through live yeast supplementation would translate
31 into altered physiology and stress tolerance. We verified how live yeast supplementation
32 affected body mass characteristics, total lipids and proteins, metabolic profiles and cold
33 tolerance (acute and chronic stress). Females fed with live yeast had increased body mass and
34 contained more lipids and proteins. Using GC/MS profiling, we found distinct metabolic
35 fingerprints according to nutritional conditions. Metabolite pathway enrichment analysis
36 corroborated that live yeast supplementation was associated with amino acid and protein
37 biosyntheses. The cold assays revealed that the presence of dietary live yeast greatly
38 promoted cold tolerance. Hence, this study conclusively demonstrates a significant interaction
39 between nutritional conditions and thermal tolerance.

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53 ## 1. Introduction

54 A number of studies have examined how nutrition affects various traits in insects, with 55 special emphasis on the effects of dietary intake on reproduction and longevity (Le Bourg and 56 Medioni, 1991; Chippindale et al., 1993; Leroi et al., 1994; Anagnostou et al., 2010). More 57 specifically, the trade-off between reproduction and lifespan resulting from the qualitative and 58 quantitative manipulations of diet has been studied extensively (Carey et al., 2008; Ellers et 59 al., 2011; Moore and Attisano, 2011). *Drosophila melanogaster* is a very popular model used 60 in the dietary restriction (DR) literature of gerontology because of its relatively short 61 generation time and ease of handling for demographic analysis (Partridge et al., 2005). The 62 composition of *Drosophila* sp. food recipes often varies among laboratories and medium 63 types, but the standard ingredients usually include water, agar, sugar, killed yeast, and 64 fungicides. In addition, the medium can be supplemented with live yeast. Often adding live 65 yeast on the surface of the diet strongly stimulates females to lay eggs (Markow and O'Grady, 66 2006; Stocker and Gallant, 2008). Dietary yeast is a major source of nutrition for the adults 67 and larvae of most saprophagous *Drosophila* (Diptera: Drosophilidae) (Begon, 1982). It 68 provides essential nutrients for the developmental and reproductive processes such as amino 69 acids, sterols, vitamins, and fatty acids (Davis, 1975; Anagnostou et al. 2010). As a result, the 70 concentration of yeast in the artificial diet is the primary determinant of egg production in *D.* 71 *melanogaster* (Sang and King, 1961; Skorupa et al., 2008), and the formation of yolk proteins 72 can thus be strongly curtailed by depriving flies of nutritional proteins or essential amino acid 73 present in yeasts (Sang and King 1961; Bownes et al., 1988; Chippindale et al., 1993). Hence, 74 the incorporation of live yeast in mediums highly stimulates vitellogenesis because it provides 75 essential nutrients that are not necessarily present in large amounts in the usual adult food 76 (Sang and King, 1961; Simmons and Bradley, 1997).

77 Owing to the major importance of the nutritional status on physiological and 78 biochemical processes of insects, any alteration of nutritional regime is likely to affect all 79 aspects of their life, including not only reproduction but also stress tolerance (such as thermal 80 tolerance) (Hallman and Denlinger, 1998; Chown and Nicolson, 2004; Nyamukondiwa and 81 Terblanche, 2009; Andersen et al., 2010; Colinet and Boivin, 2011; Sisodia and Singh, 2012). 82 Yet, a limited number of studies have considered the impact of nutritional resources on 83 environmental stress tolerance, and more particularly on thermal tolerance (Andersen et al., 84 2010). In this particular respect, no clear-cut response has been observed as nutritional effects 85 on thermal tolerance seem to be rather complex and involve many interacting factors.

86 Carbohydrate-rich diets tend to increase drosophilids' cold tolerance compared to protein-rich
87 diets, and the opposite effect is observed on measures of heat resistance (Andersen et al.,
88 2010; Sisodia and Singh, 2012). However, when supplemented at high levels, dietary sugars
89 induce a severe nutritional imbalance and a pathological state in *D. melanogaster* (Wang and
90 Clark, 1995; Skorupa et al., 2008; Musselman et al., 2011; Colinet et al., 2013a) and these
91 high sugar doses negatively affect cold tolerance (Colinet et al., 2013a). Quantitative
92 manipulation of food supply *via* dietary restriction (*i.e.* dilution.) has no detectable effect on
93 cold tolerance (chill-coma recovery, CCR) of young flies and only marginally reduces cold
94 tolerance later in adult life (Burger et al., 2007). Removing (or adding) live yeast from *D.*
95 *melanogaster* food also impacts on thermal traits in a rather complex manner. Le Rohellec
96 and Le Bourg (2009) found that removing live yeast only weakly decreased cold survival of
97 females subjected to a 16 h coldshock (0 °C), but only when these were mated. In another
98 study, absence of live yeast in food killed nearly 100% of flies (males and females) subjected
99 to the same cold treatment, whereas access to live yeast resulted in medium to high survival
100 rate depending on age (Le Bourg, 2010). Tolerance to heat (37 °C) was either unaffected (Le
101 Bourg, 2010) or improved by removal of live yeast (but in young females only) (Le Rohellec
102 and Le Bourg, 2009). From the above examples, it seems clear that nutritional status can be a
103 significant component of thermal tolerance of insects, affecting both heat- and cold-related
104 traits. It also appears that nutritional effects on thermal tolerance depend on several
105 interacting factors including gender, mating status, and age. Although the physiological and
106 biochemical basis of thermal responses is becoming clearer through metabolic and
107 physiological studies (Overgaard et al., 2007; Doucet et al., 2009; Colinet et al., 2012a; Kostál
108 et al., 2012; Storey and Storey, 2012; Teets and Denlinger 2013), there remains limited
109 information on the physiology of nutrition-mediated variation in thermal tolerance. A way in
110 which insects deal with nutrient variations is through altered physiology, namely by affecting
111 developmental and metabolic processes (Markow et al., 1999). Therefore, it can be assumed
112 that manipulating the source of essential nutrients found in live yeast, such as amino acids and
113 proteins, could alter the physiology and also the general stress tolerance.

114 In the present study, we completed a comprehensive assessment of the impact of
115 dietary live yeast supplementation on body mass characteristics, proteins, metabolic profiles
116 and basal cold tolerance (to acute and chronic exposures) in *D. melanogaster* females. We
117 hypothesized that the absence of the source of proteins (*i.e.* live yeast) from adult food would
118 be associated with deep physiological alterations; therefore, we expected contrasted metabolic

119 profiles (*i.e.* metabotype) between yeast-deprived and yeast-fed females. Because live yeast is
120 a rich source of proteins and amino acids, we hypothesized that pathways related to protein
121 biosynthesis would be particularly targeted by dietary live yeast supplementation. In addition,
122 we expected body mass parameters to be strongly curtailed by depriving females of live yeast.
123 Finally, we hypothesized that the nutritional and the metabolic variations caused by
124 manipulating dietary live yeast will translate into altered thermal stress tolerance.

125

126 ## 2. Materials and methods

127 ### 2.1. Fly culture and diets

128 We conducted the experiments on a mass-bred *D. melanogaster* line derived from the
129 mix of two wild populations collected in October 2010 and September 2011 at Plancoët
130 (Brittany, France). Prior to the experiment, flies were maintained in laboratory in 200 mL
131 bottles at 25 ± 1 °C (16L:8D) on standard fly medium consisting of deactivated brewer yeast
132 (80 g/L) (MP Biochemicals, Illkirch, France), sucrose (50 g/L), agar (15 g/L), kalmus (9 g/L)
133 and Nipagin® (8 mL/L) as described previously (Colinet et al., 2013a). To generate flies for
134 the experiments, groups of 15 mated females were allowed to lay eggs during a restricted
135 period of 6 h in bottles (200 mL) containing 25 mL of standard fly medium. This controlled
136 procedure allowed larvae to develop under uncrowded conditions at 25 ± 1 °C (16L:8D). At
137 emergence, adult flies were allowed to age for six days on different diets and controls. The
138 diets were changed every day for six consecutive days. Two different experiments were used
139 to assess the effect of adult dietary live yeast supplementation (see Fig. 1 for experimental
140 design).

141 - **<u>Experiment 1</u>** (conducted in 2012): minimal control diet *versus* live yeast-
142 supplemented diet. Sugar and agar [**SA**] *versus* sugar, agar, live yeast [**SAY(+)**].
143 - **<u>Experiment 2</u>** (conducted in 2013): standard control diet *versus* live yeast-
144 supplemented diet. Sugar, agar, killed yeast [**SAY(-)**] *versus* sugar, agar, killed yeast
145 and live yeast [**SAY(±)**].

146

147 In the first experiment, emerging flies did not have any nutrient supply except from sugar. It
148 is thus conceivable that these flies could suffer from malnutrition. Therefore, a second

149 experiment was designed with a standard diet as control that contains protein supply [SAY(-)]
150 rather than a minimal diet [SA], in order to assess the effect of dietary live yeast
151 supplementation without any putative malnutrition. In both experiments, the amounts of
152 sugar, agar and killed yeast when supplied were 50 g/L, 15 g/L and 80 g/L respectively. When
153 supplemented, the live yeast was provided with *ad libitum* paste placed on the surface of the
154 food [*i.e.* for SAY(+) and SAY(±)]. We used synchronized six day-old adults for all assays to
155 avoid the uncontrolled variation of stress tolerance during the first days of age (Colinet et al.,
156 2013b). Adults were sexed visually (with an aspirator) without CO_2 to avoid any confusing
157 metabolic effects due to anaesthesia (Colinet and Renault, 2012), and only females were kept.
158 Six day-old females from each nutritional group were either directly used for the cold assays
159 or snap-frozen in liquid nitrogen and stored at -80 °C for the other assays.

160

161 **2.2. Body mass and protein levels**

162 We assessed total protein content using the Bradford procedure (Bradford, 1976).
163 Twelve biological replicates, each consisting of a pool of three females, were used for each
164 experimental condition. Each sample was *vacuum*-dried (GENEVAC, model DNA-23050-
165 B00) set at 30 °C for 24 h and then weighed (dry mass, Mettler Toledo UMX2, accurate to 1
166 µg) before proteins were extracted in a phosphate buffer (100 mM KH_2PO_4, 1 mM DTT and 1
167 mM EDTA, pH 7.4, Foray et al., 2012) and homogenized using bead-beating at 25 Hz for 1.5
168 min. The concentration of total proteins was then measured in the whole body extracts using
169 Bio-Rad Protein Assay (catalog number 500-0006) following manufacturer's instructions.

170 For each nutritional treatment, 15 females were subjected to individual fresh mass
171 (FM) measurements (Mettler Toledo UMX2, accurate to 1 µg). Then, individual females were
172 dried at 60 °C for two days, and reweighed to measure dry mass (DM). Water mass (WM)
173 was determined by subtracting DM from FM. Water content (WC) represents the ratio
174 WM/FM. Lean dry mass (LDM) was measured by extracting total lipids in a
175 chloroform/methanol solution (Folch reagent 2:1, v:v) for one week under continuous
176 agitation. The samples were then dried at 60 °C to eliminate residues of the extracting
177 solution before measurement of LDM. Body lipid mass (LM), corresponding to DM-LDM,
178 was calculated (see Colinet et al., 2006). Folch reagent may extract a small fraction of other

179 compounds than lipids, but measurements obtained with this method are still considered as a
180 good index of lipid content for comparative studies (Williams et al., 2011).

181

182 ## 2.3. **Cold tolerance assays**

183 Different metrics were used to assess cold tolerance. First, recovery time following a
184 non-lethal chronic cold stress was measured (*i.e.* chill-coma recovery, CCR). Fifty females
185 were exposed to 0 °C for various durations: 8, 10 and 12 h for the flies of the experiment 1
186 [*i.e.* SA *vs.* SAY(+)], and 10 and 12 h for the flies of the experiment 2 [*i.e.* SAY(-) *vs.*
187 SAY(±)]. Cold-exposed flies were then allowed to recover at 25 ± 1 °C (16L:8D) and
188 recovery times were individually recorded; flies were considered recovered when they stood
189 up. A cold incubator (Model MIR-153, SANYO Electric Co. Ltd, Japan) was used for the
190 assays. After scoring the recovery times, the same females were returned to 25 ± 1 °C
191 (16L:8D) on their respective diet and the mortality was scored after 24 h (*i.e.* latent damage
192 assessment).
193 Second, tolerance to acute cold stress was measured. A total of 100 females (5 replicates, 20
194 females per replicate) were placed in 42 mL glass vials immersed in a glycol solution cooled
195 to -3.5 °C for different durations: 90, 120 and 135 min for the flies of the experiment 1 [*i.e.*
196 SA *vs.* SAY(+)], and 90 and 120 min for the flies of the experiment 2 [*i.e.* SAY(-) *vs.*
197 SAY(±)]. After the acute cold stress, the flies were returned to 25 °C on their respective diet,
198 and the mortality was scored after 24 h. Most mortality in *D. melanogaster* adults happens
199 within 24 h after the cold stress (Rako and Hoffmann, 2006), and we therefore did not
200 consider a longer period.

201

202 ## **2.4. Metabolic fingerprinting**

203 The metabolic effect of dietary live yeast supplementation was assessed by comparing the
204 metabotypes of SA *vs.* SAY(+) (experiment 1) and SAY(-) *vs.* SAY(±) (*i.e.* experiment 2). To
205 ensure that the differences observed were not only related to presence/absence of live yeast in
206 the gut content, we included an additional treatment where flies were starved before sampling.
207 In this **experiment 3** (conducted in 2013), the same flies as in the experiment 2 were starved
208 for 8 h on agar before their metabolic profiles were compared. Hence, we compared the

209 following conditions: sugar, agar, killed yeast, plus 8h starvation (**St-SAY-**) *versus* sugar,
210 agar, killed yeast and live yeast, plus 8h starvation (**St-SAY±**) (see Fig. 1).

211 For each nutritional group, six biological replicates, each consisting of a pool of 15
212 females, were used for metabolic fingerprinting. Each sample was weighed (Mettler Toledo
213 UMX2, accurate to 1 µg) before metabolite extractions. Sample preparation and derivatization
214 were performed as previously described (Colinet et al., 2012b), with minor modifications.
215 Briefly, after homogenisation in methanol-chloroform solution (2:1, v:v) and phase separation
216 with 400 µL of ultrapure water, an 120 µL aliquot of the upper phase, which contained polar
217 metabolites, was *vacuum*-dried. The dry residue was resuspended in 30 µL of 20 mg mL^{-1}
218 methoxyamine hydrochloride in pyridine before incubation under automatic orbital shaking at
219 40 °C for 60 min. Then, 30 µL of MSTFA were added and the derivatization was conducted
220 at 40 °C for 60 min under agitation (see Colinet et al., 2012b). A CTC CombiPal autosampler
221 (GERSTEL GmbH and Co.KG, Mülheim an der Ruhr, Germany) was used, ensuring
222 standardized sample preparation and timing. Metabolites were separated, identified and
223 quantified using a GC/MS platform consisting of a Trace GC Ultra chromatograph and a
224 Trace DSQII quadrupole mass spectrometer (Thermo Fischer Scientific Inc, Waltham, MA,
225 USA). The oven temperature ranged from 70 to 170 °C at 5 °C min^{-1}, from 170 to 280 °C at 7
226 °C min^{-1}, from 280 to 320 °C at 15 °C min^{-1}, and then, the oven remained at 320 °C for 4 min.
227 We completely randomized the injection order of the samples. All samples were run under the
228 SIM mode rather than the full-scan mode. We therefore only screened for the 63 pure
229 reference compounds included in our custom spectral database. Calibration curves for 60 pure
230 reference compounds at 5, 10, 20, 50, 100, 200, 500, 750, 1000, 1500 and 2000 µM
231 concentrations were run concurrently. Chromatograms were deconvoluted using XCalibur
232 2.0.7, and metabolite levels were quantified using the quadratic calibration curve for each
233 reference compound and concentration. Arabinose was used as the internal standard (see
234 Colinet et al., 2012b). Among the 63 metabolites included in our spectral library, 37, 34 and
235 34 compounds were detected in the samples from experiment 1, 2 and 3 respectively (see
236 Table 1 for compounds' list and abbreviations).
237

238 ## 2.5. Statistics

239 Since allometric relationship may exist between the body mass parameters and size, we
240 first determined if the variables were linearly related to LDM (with least-squares regressions)

241 (Packard and Boardman, 1999). Analysis of covariance (ANCOVA) was then used if linear
242 relationships was established, using LDM as co-variable, whereas analysis of variance
243 (ANOVA1) was used with nutritional treatment as factor when the allometric relationship was
244 not found. The same approach was used for analysing the protein content but with DM as co-
245 variable. Comprehensive details on regression statistics and individual plots are shown in
246 supplementary file S1. For cold tolerance, Chi-square contingency tests were used to compare
247 mortality rates between nutritional groups (with Yates' correction to prevent overestimation of
248 statistical significance). For CCR, the data were used to generate temporal recovery curves
249 which were compared with Mantel-Cox (Log rank) test. This non parametric method tests the
250 null hypothesis that there is no difference between the populations in the probability of an
251 event at any time point (i.e. a curve comparison test). Analyses were performed using Prism v.
252 5.01 (GraphPad Software, Inc., San Diego, CA, USA, 2007) or the statistical software 'R
253 2.13.0' (R Development Core Team, 2008). For metabolic data, a principal component
254 analysis (PCA) was performed on the whole dataset to detect the compounds contributing the
255 most to the separation between the nutritional groups. The inertia calculated in the PCA
256 represents the part of the total variance that is due to the difference between modalities.
257 Scaled data (*i.e.* mean-centered and divided by $\sqrt{SD}$) were used in the multivariate analyses to
258 prevent the effects of the metabolite concentration means and ranges of variability on the
259 correlations with the principal components (PCs). This analysis was performed using the
260 'ade4' library in the statistical software 'R 2.13.0'. In addition, to look for evidence of enriched
261 metabolic pathways in response to dietary live yeast supplementation, metabolite pathway
262 enrichment analysis (MPEA) was conducted using MetPA online package, with *D.*
263 *melanogaster* specific library (Xia and Wishart, 2010), as previously described (Colinet et al.,
264 2013a).
265

266 # 3. Results

267 ## 3.1. Body mass and protein levels

268 The Figure 2 summarizes the variations in mass parameters according to nutritional
269 treatments. The DM corresponds to the sum of LM and LDM, and FM corresponds to the sum
270 of LM, LDM and WM (Fig. 2). Females fed with live yeast [SAY(+) and SAY(±)] were
271 heavier in terms of FM and DM than their counterparts fed without live yeast. Since FM and

272 DM were linearly related to LDM ($P < 0.05$; see supplementary file S1), we used ANCOVA
273 to assess the effect of nutritional treatment with LDM as co-variable. The effect of the
274 treatment remained significant even when the allometric effect of size was removed (FM: F =
275 30.69, df = 3, $P < 0.001$; DM: F = 8.27, df = 3, $P < 0.001$; n = 15). Multiple comparisons
276 revealed that SA < SAY(-) < SAY(+) = SAY(±) for FM, and SA < SAY(-) = SAY(+) =
277 SAY(±) for DM. The WM was also correlated to LDM ($P < 0.05$; see supplementary file S1).
278 The ANCOVA revealed a significant effect of the treatment (F = 29.94, df = 3, $P < 0.001$; n =
279 15). Multiple comparisons revealed that SA < SAY(-) < SAY(+) = SAY(±) for WM. Contrary
280 to WM, the WC was unrelated to LDM ($P > 0.05$; see supplementary file S1) and the
281 ANOVA did not detect variation according to the diet treatment (F = 0.197, df = 3, P = 1.61;
282 n = 15). The LM was unrelated to LDM ($P > 0.05$; see supplementary file S1), and ANOVA
283 detected a significant effect of nutritional treatment, with a lower total lipid content for the
284 treatment SA (F = 10.4, df = 3, $P < 0.001$; n = 15). The LDM varied according to nutritional
285 treatment (F = 76.14, df = 3, $P < 0.001$; n = 15), with the following rank order: SA < SAY(-)
286 < SAY(+) < SAY(±). Finally, the total protein content was not related to DM ($P > 0.05$; see
287 supplementary file S1), and ANOVA revealed a significant effect of nutritional treatment (F =
288 155.3, df = 3, $P < 0.001$; n = 12), with higher protein contents in females fed with live yeast
289 [*i.e.* SA < SAY(-) < SAY(+) < SAY(±)].
290

291 ## 3.2. Cold tolerance

292 Concerning cold tolerance, we found that CCR significantly varied between the two
293 nutritional groups of the experiment 1, with females fed on SAY(+) diet recovering faster
294 than females fed on SA diet (Fig. 3). This difference manifested for all the durations of cold
295 stress that were tested in the experiment 1 (8h: Chi^2 = 19.17, df = 1, $P < 0.001$; 10 h: Chi^2 =
296 16.29, df = 1, $P < 0.001$; 12 h: Chi^2 = 14.65, df = 1, $P < 0.001$; n = 50). Survival after chronic
297 cold stress was also affected by nutritional regime. For all the durations of cold stress at 0 °C
298 (8, 10 and 12 h), the post-stress mortality was significantly lower when females fed on
299 SAY(+) diet compared to SA diet (8 h: Chi^2 = 21.23, df = 1, $P < 0.001$; 10 h: Chi^2 = 19.10, df
300 = 1, $P < 0.001$; 12 h: Chi^2 = 21.23, df = 1, $P < 0.001$; n = 50) (Fig. 3). Finally, the acute cold
301 tolerance also varied with the nutritional regimes of the experiment 1 (Fig. 3). For all the
302 durations of cold stress at -3.5 °C (90, 120 and 135 h), the post-stress mortality was
303 significantly lower when females fed on SAY(+) diet compared to SA diet (90 min: Chi^2

304 =39.61, *df* = 1, $P < 0.001$; 120 min: Chi^2 =35.57, *df* = 1, $P < 0.001$; 135 min: Chi^2 =17.56, *df* =
305 1, $P < 0.001$; $n = 100$).

306 The cold tolerance of the flies from the experiment 2 was also affected by the
307 nutritional treatments; however, this was not manifested on CCR. Females feeding on SAY(-)
308 and SAY(±) recovered from chronic cold stress with similar temporal dynamics (10 h: Chi^2 =
309 2.55, *df* = 1, $P = 0.10$; 12 h: Chi^2 = 1.01, *df* = 1, $P = 0.31$; $n = 50$) (Fig. 4). On the other hand,
310 the survival after the chronic cold stress was affected by the nutritional regimes. For both
311 durations of chronic cold stress (10 and 12 h), the post-stress mortality was significantly lower
312 when females fed on SAY(±) diet compared to SAY(-) diet (10 h: Chi^2 = 5.02, *df* = 1, P =
313 0.025; 12 h: Chi^2 = 16.94, *df* = 1, $P < 0.001$; $n = 50$) (Fig. 4). Finally, the acute cold tolerance
314 also varied with the nutritional regimes of the experiment 2. For both durations of acute cold
315 stress at -3.5 °C (90 and 120 h), the post-stress mortality was significantly lower when
316 females fed on SAY(±) diet compared to SAY(-) diet (90 min: Chi^2 =69.01, *df* = 1, $P < 0.001$;
317 120 min: Chi^2 =66.66, *df* = 1, $P < 0.001$; $n = 100$) (Fig. 4).
318

319 **3.3. Metabolic fingerprinting**

320 The metabolic profiles of flies from experiment 1 showed that a number of metabolites
321 had their concentrations affected by dietary live yeast, which resulted in contrasted
322 metabotypes between the two nutritional groups (Fig. 5). A clear-cut separation was observed
323 along the first principal component (PC1) of the PCA, which accounted for 43.9% of the total
324 inertia (Fig. 5). GDL, Fru, Glc, Tre and sorbitol were the molecules the most positively
325 correlated to PC1 (*i.e.* accumulated in SA flies), whereas on the opposite side, the amino acids
326 Val, Ile, Leu, Thr, Gly, Phe and Glu were the molecules the most negatively correlated to PC1
327 (*i.e.* accumulated in SAY(+) flies) (Fig. 5). The other principal components accounted for
328 28.4% (PC2) and 11.3% (PC3) of the total inertia and mainly represented within-treatment
329 variations. MPEA based on the metabolites that were positively correlated to PC1 revealed
330 three enriched metabolic pathways (Holm adjust $P < 0.05$), and all were directly involved in
331 carbohydrate metabolism. MPEA based on all the metabolites that were negatively correlated
332 to PC1 revealed three enriched metabolic pathways; all were directly involved in amino acids
333 and protein biosynthesis (see Dataset S1 for detailed concentrations and fold changes).

334 Similar results were obtained with the flies from experiment 2. A clear-cut separation
335 was observed along the PC1 of the PCA, which accounted for 47.1% of the total inertia (Fig.
336 6). Fru, Tre, xylitol, Glc and GDL were the most positively correlated to PC1 (*i.e.*
337 accumulated in SAY(-) flies), whereas on the opposite side, Glu, inositol, Leu, Phe and Val
338 were the most negatively correlated metabolites to PC1 (*i.e.* accumulated in SAY(±) flies)
339 (Fig. 6). The other principal components accounted for 31.6% (PC2) and 6.4% (PC3) of the
340 total inertia and mainly represented within-treatment variations. MPEA also revealed that that
341 carbohydrate metabolism was enriched in the SAY(-) flies, while amino acids and protein
342 biosynthesis were enriched in the SAY(±) flies (see Dataset S2 for detailed concentrations and
343 fold changes).

344

345 Finally, the addition of a starvation period to empty the gut content of the flies before
346 assessing the flies (*i.e.* experiment 3) resulted in a similar metabolic response. Again, a clear-
347 cut separation was observed along the PC1, which accounted for 57.3% of the total inertia
348 (Fig. 7). Xylitol, Man, Ala, Fru, Tre and Glc were the most positively correlated metabolites
349 to PC1 (*i.e.* accumulated in St-SAY(-) flies), whereas Glu, Thr, Ile, Phe, inositol and Leu
350 were the most negatively correlated to PC1 (*i.e.* accumulated in SAY(±) flies) (Fig. 7). The
351 other principal components accounted for 21.2% (PC2) and 7.15% (PC3) of the total inertia.
352 MPEA also revealed that that carbohydrate metabolism was enriched in the St-SAY(-) flies,
353 while amino acids and protein biosynthesis were enriched in the St-SAY(±) flies (see Dataset
354 S3 for detailed concentrations and fold changes).

355

356 ## 4. Discussion

357 Dietary yeast is a major source of nutrition for the adults and larvae of most
358 saprophagous *Drosophila* sp. (Diptera: Drosophilidae) (Begon, 1982), and as a consequence,
359 yeast is typically incorporated into artificial diets (Markow and O'Grady, 2006; Stocker and
360 Gallant, 2008). Dietary yeast provides essential nutrients such as amino acids, sterols,
361 vitamins, and fatty acids (Davis, 1975; Anagnostou et al., 2010). We assumed that removing
362 or adding live yeast from adult food at eclosion would be associated with physiological
363 remodelling that would subsequently affect fitness-related traits such as body size and stress
364 tolerance. In the present study, we completed a comprehensive assessment of the impact of

365 dietary live yeast supplementation on body mass characteristics, stored proteins, metabolic
366 profiles and basal cold tolerance (to acute and chronic exposures) in *D. melanogaster* females.

367 We expected body mass parameters to be affected by dietary live yeast
368 supplementation. Indeed, the body mass of the flies is known to reflect protein level in food,
369 with high levels of dietary yeast leading to heavier flies (Skorupa et al., 2008). We have
370 conclusively shown that body mass parameters (FM, DM, WM, LM and LDM) increased
371 when females were fed with live yeast, which is consistent with previous studies (Simmons
372 and Bradley, 1997; Le Rohellec and Le Bourg, 2009). For all the considered mass parameters,
373 the SA flies had significantly smaller values than the SAY(-) flies which shows that SA flies
374 disproportionally suffered from the complete lack of dietary protein and suggests a
375 malnutrition in this group. Concerning the fat (*i.e.* LM), we found that the SA flies had lower
376 stored fat than the live yeast-fed flies [*i.e.* SAY(+), SAY(±)], but this reduction was not
377 observed in SAY(-) flies. Hence, the reduction of fat was not related to the suppression of live
378 yeast *per se*, but to the complete suppression of proteins supply from the diet (*i.e.* SA). It was
379 previously reported that the increase in body mass with dietary live yeast is almost
380 exclusively due to increased ovary size (Simmons and Bradley, 1997), and ovaries comprise
381 approximately 15% of the body lipids of insects (Lease and Wolf, 2011). The lower LM of
382 the flies on SA diet is not surprising as these flies had small ovaries and hardly produced eggs
383 (data not shown). The LDM was different among all treatments and a corresponding pattern
384 was observed for the protein content. This suggests that feeding on a diet that contains killed
385 yeast [SAY(-)] provides proteins to the flies, but feeding on a diet that also contains live yeast
386 provides additional amounts of proteins. Storage of proteins is largely independent of dietary
387 carbohydrates but is almost exclusively determined by the presence and concentration of yeast
388 in the medium (Skorupa et al., 2008). Our data corroborate this idea.

389 A way in which insects deal with nutrient variations is through altered physiology,
390 namely by affecting developmental and metabolic processes (Markow et al., 1999). Therefore,
391 we assumed that manipulating the adult food (*via* live yeast supplementation) would be
392 associated with physiological changes that would translate into contrasted metabolic profiles
393 between nutritional groups. We have conclusively shown that a number of metabolites had
394 their concentrations affected by the nutritional treatments, which resulted in contrasted
395 metabotypes between live yeast-supplemented flies [SAY(+) and SAY(±)] and the control
396 flies. Whatever the control used [SA or SAY(-) or St-SAY(-)], a similar response was
397 repeatedly observed : sugars (Fru, Glc, and Tre) exhibited elevated amounts in the control

398 whereas amino acid amounts (Val, Ile, Leu, Thr, Gly, Phe and Glu) were more abundant in
399 the live yeast-supplemented groups. The fact that the relative abundance of sugars was higher
400 in SA metabotype is not surprising, as these flies were fed on a minimal diet with no access to
401 any source of proteins from adult eclosion. For the flies fed on SAY(-) and St-SAY(-) diets,
402 the increased levels of sugars likely translates that these diets were proportionally richer in
403 sugar than the corresponding live yeast-supplemented diets. We also found that GDL, sorbitol
404 and xylitol contributed to the control metabotypes. GDL is a metabolite (a lactone) resulting
405 from the degradation of Glc through the pentose phosphate pathway (Garrett and Grisham,
406 1999). Polyols such as sorbitol are derived from hexose monophosphates and can be produced
407 from both Glc and Fru (Storey, 1983; Wolfe et al., 1998). The higher relative abundance of
408 these sugar-related compounds is thus congruent with the nutritional regime of the flies.
409 Moreover, MPEA revealed several enriched metabolic pathways associated with the control
410 metabotypes [SA or SAY(-) or St-SAY(-)], and all of them were directly involved in the
411 carbohydrate metabolism. This further confirmed the relative higher impact of sugars in
412 shaping the metabotype of these nutritional groups. Concerning the live yeast-fed flies, we
413 found a higher relative abundance of amino acids (*e.g.* Val, Ile, Leu, Thr, Gly, Phe and Glu)
414 associated with these nutritional groups. This response was observed whatever the treatment
415 used [SAY(+) or SAY(±), or St-SAY(±)]. This is congruent with the nutritional regime of
416 these flies. Live yeast is known to provide essential nutrients such as proteins and amino acids
417 (Davis 1975; Anagnostou et al. 2010). This most likely explains why MPEA revealed several
418 enriched metabolic pathways related to amino acids and protein biosynthesis in these
419 nutritional groups. This biological interpretation also coincides with the larger body protein
420 content detected in these nutritional groups. The differences observed in the metabolic
421 profiles between the live yeast-supplemented and the control groups may also be partly due to
422 different food intake and thus incorporation of nutrients. Indeed, food intake increases with
423 concentration of dietary yeast in *D. melanogaster* (Min and Tara, 2006). The fact that
424 metabolic patterns were consistent among experiments suggest that (i) live yeast promotes
425 amino acids biosynthesis even when the flies are already fed with killed yeast, and (ii) that
426 differences observed were not related to presence/absence of live yeast in the gut content.

427 Many insect species feed on yeasts and the effects of this nutritional resource on the
428 growth, fecundity and survival has been demonstrated in a wide range of species (*e.g.*
429 Starmer and Fogleman, 1986; Ganter 2006; Anagnostou et al. 2010). In spite of this, there is
430 limited information on nutrition-mediated variations in stress tolerance in insects, and more

431 particularly regarding thermal tolerance (Andersen et al., 2010). Here, we report convincing
432 evidence that supplementing adult flies with sources of dietary proteins and amino acids
433 (live yeast) promoted their subsequent cold tolerance. This positive effect of live yeast was
434 repeatedly found in almost all of the metrics used to assess their cold tolerance (acute and
435 chronic tolerance), and for all the stress intensities applied in the first experiment. In the
436 second experiment, CCR was not affected by live yeast supplementation, but all the other
437 assays (post-stress survival) supported a positive effect of live yeast on cold tolerance.
438 Previous works reported an effect of dietary yeast on *Drosophila* cold tolerance, but the
439 effects ranged from weak to very intense, and were thus difficult to interpret. For instance,
440 Le Rohellec and Le Bourg (2009) found that removing live yeast weakly decreased cold
441 survival of females subjected to a 16h cold-shock (0 °C), but only when these were mated.
442 In another study, the absence of live yeast in food killed nearly all flies (males and females)
443 subjected to the same cold treatment, whereas access to dietary yeast resulted in medium to
444 high survival rates, depending on the age of the specimens (Le Bourg, 2010). These
445 incongruities likely arise from the fact that nutrition-related variation in thermal tolerance
446 involves interacting factors such as age, mating and gender. It remains unclear why in our
447 study the CCR was affected by the nutritional treatment in the experiment 1 but not in the
448 experiment 2. Longer temporal recovery dynamics of the SA flies (experiment 1) could
449 result from the lack of essential nutrients necessary for an optimal functioning of the whole-
450 system physiology, or from an excessive amount of consumed sugars (as sugar was the sole
451 source of food in this specific group). With regard to sugars, it appears that carbohydrate-
452 enriched diets tend to increase *Drosophila* cold tolerance (Andersen et al., 2010; Sisoda and
453 Singh, 2012). However, when provided at too high levels, dietary sugars induce a severe
454 nutritional imbalance and a pathological state in *D. melanogaster* (Wang and Clark, 1995;
455 Skorupa et al., 2008; Musselman et al., 2011; Colinet et al., 2013a), which in turn negatively
456 affects cold tolerance including CCR (Colinet et al., 2013a). In spite of this, our data and
457 earlier observations (Le Rohellec and Le Bourg, 2009; Le Bourg, 2010) all converge
458 towards the same conclusion that cold tolerance of the females of *D. melanogaster* is
459 generally promoted by dietary live yeast. Females fed with live yeast had increased body
460 mass and contained more lipids and proteins, and MPEA corroborated that live yeast
461 supplementation was associated with amino acids and protein biosyntheses. Interestingly, it
462 was previously found in *D. melanogaster* that the level of glycogen, triglycerides, and total
463 proteins was higher in cold-selected than in control lines (Chen and Walker, 1994). The
464 same authors also noted that these levels quickly decreased 24 h after a cold stress and

465 suggested that higher storage of energy reserves entails increased cold tolerance of cold-
466 selected lines. Thus, the higher energy reserves of the live yeast-supplemented flies may
467 explain why cold survival (assessed after 24 h) was higher in this nutritional group.

468 Stressful conditions are known to critically increase energy expenditure because the
469 repairing mechanisms require excess of energy (Parsons, 1991). We suggest that in nutrient-
470 unbalanced conditions (*e.g.* SA), individuals might disproportionately suffer from stressful
471 conditions because the metabolically available energy is already constrained. The ability to
472 synthesize essential stress-related proteins, due to dietary depletion of amino acids and
473 protein building blocks could be an alternate explanation for the reduced cold tolerance.
474 Hence, dietary balance is likely to be a key point of environmental stress physiology. Stress
475 tolerance is probably compromised under conditions of excessive nutritional imbalance, as
476 for life-history traits (Skorupa et al., 2008). In the natural environment, larvae may
477 occasionally face nutritional stress and this might further affect the stress tolerance of the
478 adults (carry-over effect), however, this question has not been examined. This study
479 conclusively demonstrates an interaction between dietary live yeast and thermal stress
480 tolerance of *D. melanogaster* females. Whether dietary live yeast positively affects the
481 tolerance to other stressors remains to be further examined. Moreover, the mechanistic link
482 between thermal tolerance and dietary live yeast remains an open question. Understanding
483 the link between thermal stress tolerance and nutrient quality represents an important step in
484 physiological ecology that may further add to our understanding of thermal biology of
485 ectotherms.

486

487 ## 5. Acknowledgments

488 This study was supported by Observatoire des Sciences Universelles Rennais (OSUR). We
489 are grateful to Raphael Bical for technical help and to anonymous referees for helping
490 improving the manuscript

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495 **Reference**

496

497 Anagnostou, C., Dorsch, M. and Rohlfs, M., 2010. Influence of dietary yeasts on *Drosophila*
498 *melanogaster* life-history traits. Entomol. Exp. Appl. 136, 1-11.
499 Andersen, L.H., Kristensen, T.N., Loeschcke, V., Toft, S., Mayntz, D., 2010. Protein and
500 carbohydrate composition of larval food affects tolerance to thermal stress and
501 desiccation in adult *Drosophila melanogaster*. J. Insect Physiol. 56, 336-340.
502 Begon, M. 1982. Yeasts and *Drosophila*. In: Ashburner, M., Carson H.L., Thompson J.
503 (Eds.), The genetics and biology of *Drosophila*, Academic Press, New York, pp 345-
504 384.
505 Bownes, M., Scott, A., Shirras, A., 1988. Dietary components modulate yolk protein gene
506 transcription in *Drosophila melanogaster*. Development 103, 119-128.
507 Bradford, M.M. 1976., A rapid and sensitive method for the quantitation of microgram
508 quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72,
509 248-254.
510 Burger, J.M.S., Hwangbo, D.S., Corby-Harris, V., Promislow, D.E.L., 2007. The functional
511 costs and benefits of dietary restriction in *Drosophila*. Aging Cell 6, 63-71.
512 Carey, J.R., Harshman, L.G., Liedo, P., Müller, H.G., Wang, J.L., Zhang, Z., 2008.
513 Longevity–fertility trade-offs in the tephritid fruit fly, *Anastrepha ludens*, across dietary
514 restriction gradients. Aging Cell 7, 470-477.
515 Chen, C.P., Walker, V.K., 1994. Cold-shock and chilling tolerance in *Drosophila*. J. Insect
516 Physiol. 40, 661- 669.
517 Chippindale, A.K., Leroi, A.M., Kim, S.B., Rose, M.R., 1993. Phenotypic plasticity and
518 selection in *Drosophila* life history evolution. I. Nutrition and the cost of reproduction.
519 J. Evol. Biol. 6, 171-193.
520 Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology: Mechanisms and Patterns,
521 Oxford University Press, Oxford.
522 Colinet, H., Boivin, G., 2011. Insect parasitoids cold storage: A comprehensive review of
523 factors of variability and consequences. Biol. Control 58, 83-95.
524 Colinet, H., Renault, D., 2012. Metabolic effects of CO2 anaesthesia in *Drosophila*
525 *melanogaster*. Biol. Lett. 8, 1050-1054.

526 Colinet, H., Hance, T., Vernon, P., 2006. Water relations, fat reserves, survival, and longevity
527 of a cold-exposed parasitic wasp *Aphidius colemani* (Hymenoptera : Aphidiinae).
528 Environ. Entomol. 35, 228-236.
529 Colinet, H., Larvor, V., Laparie, M., Renault, D., 2012a. Exploring the plastic response to
530 cold acclimation through metabolomics. Funct. Ecol. 26, 711-722.
531 Colinet, H., Renault, D., Charoy-Guével, B., Com, E., 2012b. Metabolic and proteomic
532 profiling of diapause in the aphid parasitoid *Praon volucre*. PLoS One 7, e32606.
533 Colinet, H., Larvor, V., Bical, R., Renault, D., 2013a. Dietary sugars affect cold tolerance of
534 *Drosophila melanogaster*. Metabolomics. 9, 608-622.
535 Colinet, H., Siaussat, D., Bozzolan, F., Bowler, K., 2013b. Rapid decline of cold tolerance at
536 young age is associated with expression of stress genes in *Drosophila melanogaster*. J.
537 Exp. Biol. 216, 253-259.
538 Davis, G.R.F., 1975. Essential dietary amino acids for growth of larvae of the yellow
539 mealworm, *Tenebrio molitor* L. J. Nutr. 105, 1071-1075.
540 Doucet, D., Walker, V.K., Qin, W., 2009. The bugs that came in from the cold: molecular
541 adaptations to low temperatures in insects. Cell. Mol. Life Sci. 66, 1404-1418.
542 Ellers, J., Ruhe, B., Visser, B., 2011. Discriminating between energetic content and dietary
543 composition as an explanation for dietary restriction effects. J. Insect Physiol. 57, 1670-
544 1676.
545 Foray, V., Pelisson, P.F., Bel-Venner, M.C., Desouhant, E., Venner, S., Menu, F., Giron, D.,
546 Rey, B., 2012. A handbook for uncovering the complete energetic budget in insects: the
547 van Handel's method (1985) revisited. Physiol. Entomol. 37, 295-302.
548 Ganter, P.F., 2006. Yeast and Invertebrate Associations. In: Rosa, C.A., Szabo P.G. (Eds.),
549 Biodiversity and Ecophysiology of Yeasts, Springer, New York, pp 303-370.
550 Garrett, R.H., Grisham, C.M., 1999. Biochemistry. Second Edition, Saunders College
551 Publishing, Fort Worth, TX.
552 Hallman, G.J., Denlinger, D.L., 1998. Temperature sensitivity in insects and applications in
553 integrated pest management, Westview Press, USA.
554 Kostál, V., Šimek, P., Zahradníčková, H., Cimlová, J., Štětina, T., 2012. Conversion of the
555 chill susceptible fruit fly larva (*Drosophila melanogaster*) to a freeze tolerant organism.
556 Proc. Natl. Acad. Sci. USA 109, 3270-3274.
557 Lease, H., Wolf, B., 2011. Lipid content of terrestrial arthropods in relation to body size,
558 phylogeny, ontogeny and sex. Physiol. Entomol. 36, 29-38.

559 Le Bourg, E., 2010. Combined effects of suppressing live yeast and of a cold pretreatment on
560 longevity, aging and resistance to several stresses in *Drosophila melanogaster*.
561 Biogerontology 11, 245-254.
562 Le Bourg, E., Medioni, J., 1991. Food restriction and longevity in *Drosophila melanogaster*.
563 Age Nutr. 2, 90-94.
564 Le Rohellec, M., Le Bourg, E., 2009. Contrasted effects of suppressing live yeast from food
565 on longevity, aging and resistance to several stresses in *Drosophila melanogaster*. Exp.
566 Gerontol. 44, 695-707.
567 Leroi, A.M., Chippindale, A.K., Rose, M.R., 1994. Long term evolution of a life history
568 trade-off in *Drosophila melanogaster*. 1. The role of genotype by environment
569 interaction. Evolution 48, 1244-1257.
570 Markow, T , O'Grady, P.M., 2006. *Drosophila*: A guide to species identification and use,
571 Academic Press, London.
572 Markow, T.A., Raphael, B., Breitmeyer, C.M., Dobberfuhl, D., Elser, J.J., Pfeiler, E., 1999.
573 Elemental stoichiometry of *Drosophila* and their hosts. Funct. Ecol. 13, 78-84.
574 Min, K.J., Tatar, M. 2006. Drosophila diet restriction in practice: Do flies consume fewer
575 nutrients? Mech. Ageing Dev. 127, 93-96.
576 Moore, P.J., Attisano, A., 2011. Oosorption in response to poor food: complexity in the trade-
577 off between reproduction and survival. Ecol. Evol. 1, 37-45.
578 Musselman, L.P., Fink, J.L., Narzinski, K., Ramachandran, P.V., Hathiramani, S.S., Cagan,
579 R.L., Baranski, T.J., 2011. A high-sugar diet produces obesity and insulin resistance in
580 wild-type *Drosophila*. Dis. Model Mech. 4, 842-849.
581 Nyamukondiwa, C., Terblanche, J.S., 2009. Thermal tolerance in adult Mediterranean and
582 Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and
583 feeding status. J. Therm. Biol. 34, 406-414.
584 Overgaard, J., Malmendal, A., Sørensen, J.G., Bundy, J.G., Loeschcke, V., Nielsen, N.C.,
585 Holmstrup, M., 2007. Metabolomic profiling of rapid cold hardening and cold shock in
586 *Drosophila melanogaster*. J. Insect Physiol. 53, 1218-1232.
587 Packard, G.C., Boardman, T.J., 1999. The use of percentages and size-specific indices to
588 normalize physiological data for variation in body size: wasted time, wasted effort?
589 Comp. Biochem. Physiol. A 122, 37-44.
590 Parsons, P.A., 1991. Evolutionary rates: Stress and species boundaries. Annu. Rev. Ecol. Syst.
591 22, 1-18.

592 Partridge, L., Piper, M.D.W., Mair, W., 2005. Dietary restriction in *Drosophila*. Mech. Ageing Dev. 126, 938-950.
594 R Development Core Team., 2008. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, ISBN 3-900051-07-0, <http://www.Rproject.org>.
597 Rako, L., Hoffmann, A.A., 2006. Complexity of the cold acclimation response in *Drosophila melanogaster*. J. Insect Physiol. 52, 94-104.
599 Sang, J.H., King, R., 1961. Nutritional requirements of axenically cultured *Drosophila melanogaster* adults. J. Exp. Biol. 38, 793-809.
601 Simmons, F.H., Bradley, T.J., 1997. An analysis of resource allocation in response to dietary yeast in *Drosophila melanogaster*. J. Insect Physiol. 43, 779-788.
603 Sisodia, S., Singh, B.N.,2012. Experimental evidence for nutrition regulated stress resistance in *Drosophila ananassae*. PLoS One 7, e46131.
605 Skorupa, D.A., Dervisefendic, A., Zwiener, J., Pletcher, S.D., 2008. Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. Aging Cell 7, 478-490.
608 Starmer, W., Fogleman, J., 1986. Coadaptation of *Drosophila* and yeasts in their natural habitat. J. Chem. Ecol. 12, 1037-1055.
610 Stocker, H., Gallant, P., 2008. Getting started: an overview on raising and handling *Drosophila*. Methods Mol. Biol. 420, 27-44.
612 Storey, K.B., 1983. Metabolism and bound water in overwintering insects. Cryobiology 20, 365-379.
614 Storey, K.B., Storey, J.M., 2012. Insect cold hardiness: metabolic, gene, and protein adaptation. Can. J. Zool. 90, 456-475.
616 Teets, N., Denlinger, D. 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiol. Entomol. 38, 115-116.
618 Wang, L., Clark, A.G., 1995. Physiological genetics of the response to a high-sucrose diet by *Drosophila melanogaster*. Biochem. Genet. 33, 149-165.
620 Williams, C.M., Thomas, R.H., MacMillan, H.A., Marshall, K.E., Sinclair, B.J., 2011. Triacylglyceride measurement in small quantities of homogenised insect tissue: comparisons and caveats. J. Insect Physiol. 57: 1602-13
623 Wolfe, G.R., Hendrix, D.L., Salvucci, M.E., 1998. A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. J. Insect Physiol. 44, 597-603.

625 Xia, J., Wishart, D.S. 2010. MetPA: a web-based metabolomics tool for pathway analysis and
626 visualization. Bioinformatics 26, 2342-2344.
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Table 1: List of metabolites detected in females of *Drosophila melanogaster*
Compounds abbreviations in brackets

Free amino acids

- Alanine (Ala)
- Valine (Val)
- Serine (Ser)
- Leucine (Leu)
- Threonine (Thr)
- Proline (Pro)
- Methionine (Met)
- Ornithine (Orn)
- Glycine (Gly)
- Isoleucine (Ile)
- Glutamate (Glu)
- Lysine (Lys)
- Phenylalanine (Phe)
- Tyrosine (Tyr)

Sugars

- Sucrose (Suc)
- Fructose (Fru)
- Glucose (Glc)
- Trehalose (Tre)
- Mannose (Man)
- Galactose (Gal)
- Ribose (Rib)
- Maltose (Mal)
- Glucose-6-phosphate (G6P)

Polyols

- Sorbitol
- Glycerol
- Glycerol-3-Phosphate
- Inositol
- Xylitol

Intermediate metabolites

- Succinate
- Malate
- Citrate
- Fumarate

Other metabolites

- Lactate
- Ethanolamine (ETA)
- Free phosphate (PO4)
- Gamma-aminobutyric acid (GABA)
- Glucono delta-lactone (GDL)
- Spermine

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652 **Figure 1:** Schematic diagram of the experimental design used to investigate the effect of
653 dietary live yeast supplementation on mass parameters, cold tolerance and GC-MS metabolic
654 profiles of *D. melanogaster*. In all experiments, the flies developed from egg to adult on a
655 standard diet [SAY(-)]. Emerging females were then fed on different diets for 6 days: SA *vs.*
656 SAY(+) for experiment 1 and SAY(-) *vs.* SAY(±) for experiment 2. In the experiment 3,
657 females were fed on the same experimental conditions as in experiment 2, but they were
658 starved for 8 h before sampling, St-SAY(-) vs. St-SAY(±). Symbols S, A and Y for sugar,
659 agar and yeast, respectively. Sign (+), (-) and (±) for live yeast only, killed yeast only, and
660 both live and killed yeast, respectively.
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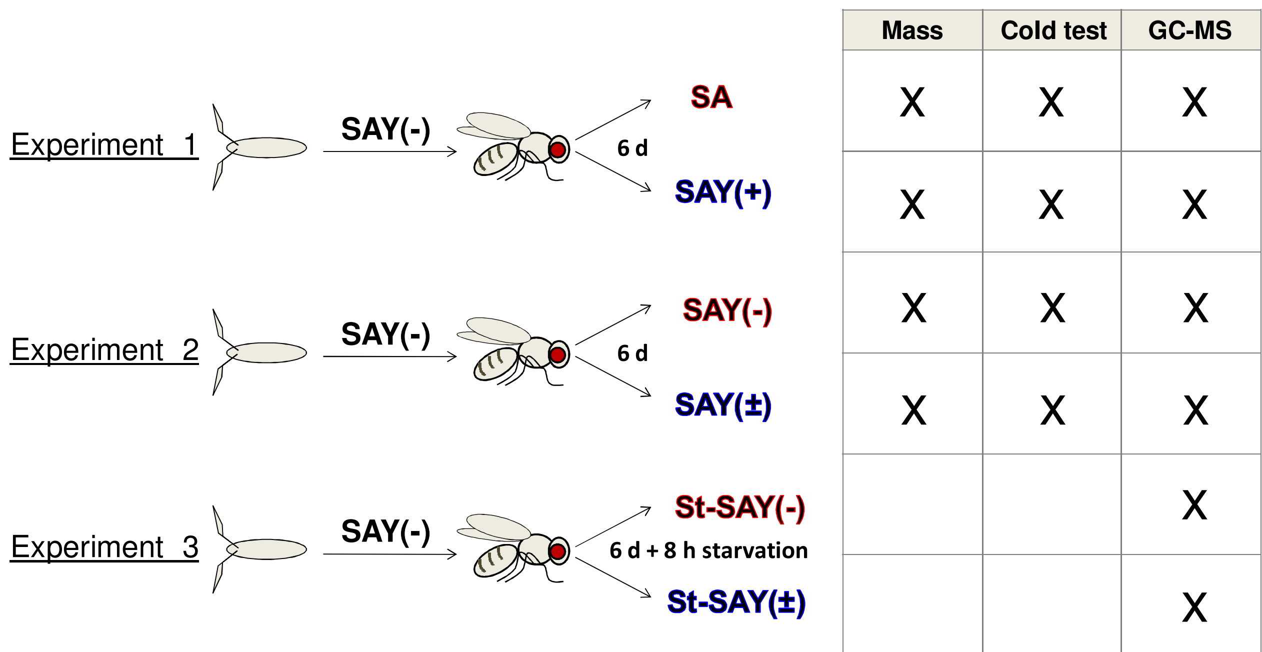

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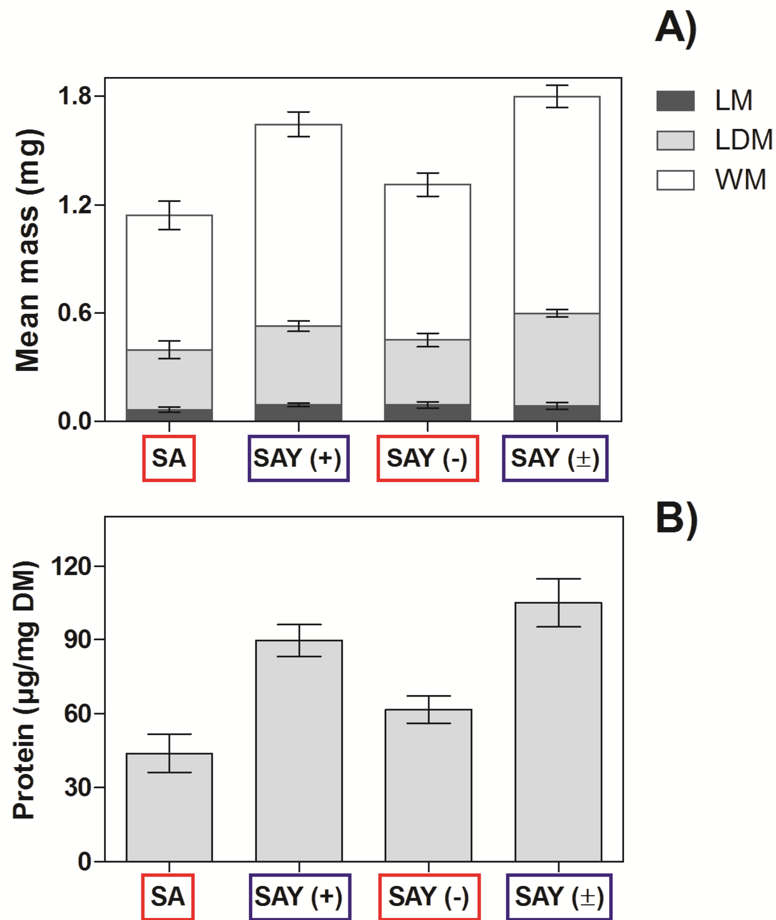
674 **Figure 2:** (A) Body mass parameters showing changes in lipid mass (LM), lean dry mass
675 (LDM), and water mass (WM) according to nutritional treatment [SA, SAY(+), SAY(-),
676 SAY(±)] (n = 15). (B) Total protein content of female *D. melanogaster* (n = 12).



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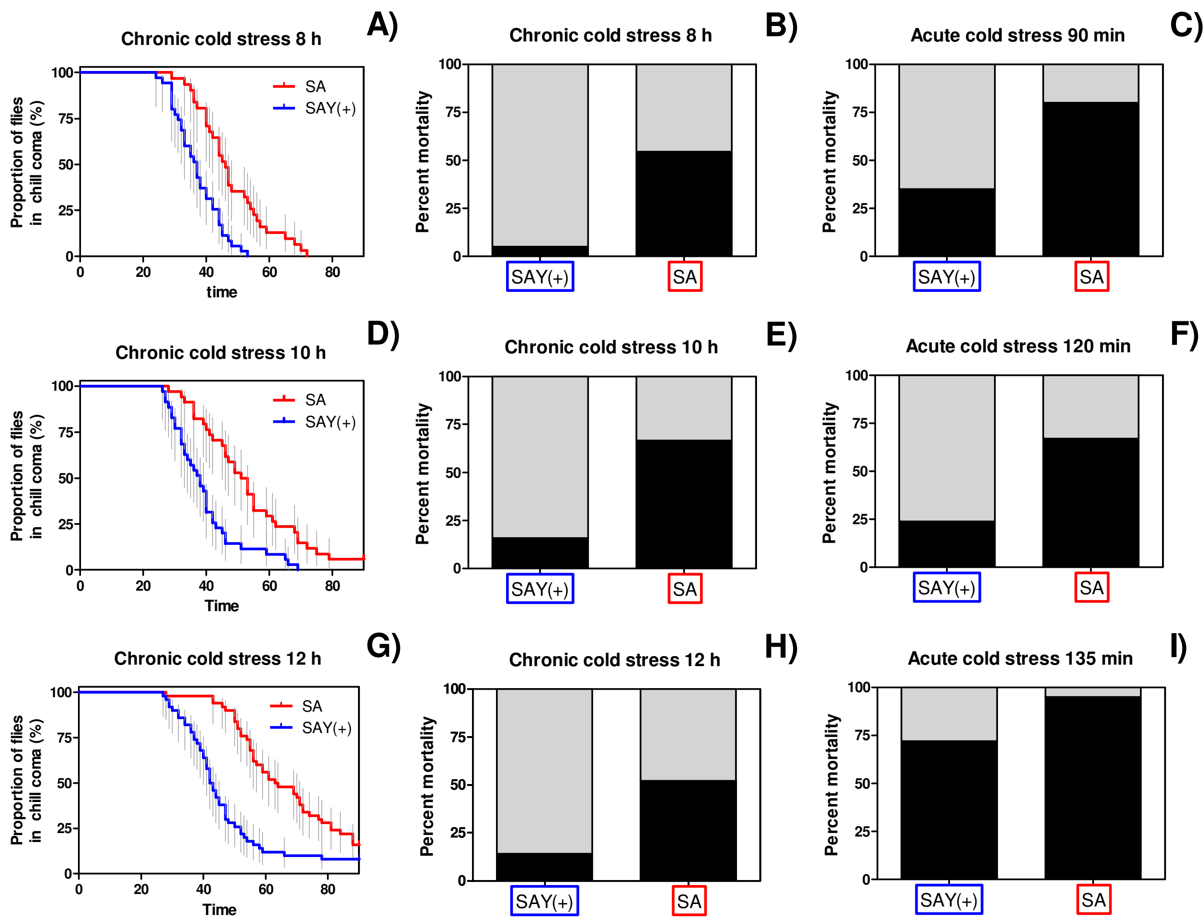
690 **Figure 3:** Composite panel summarizing all the cold tolerance assays of the experiment 1.
691 Temporal recovery curves of live yeast-fed females [blue line, SAY(+)] and yeast-deprived
692 females [red line, SA] exposed to chronic cold stress (0 °C) for various durations: 8, 10 and
693 12 h in figures A, D, and G, respectively. Each line represents the mean proportion (± 95%
694 confidence interval) of recovering flies in relation to time after cold stress (*n* = 50). Mortality
695 rates, assessed 24 h after the chronic cold stresses, are shown in figures B, E and H for each
696 nutritional treatment [SA *vs.* SAY(+)] (*n* = 50). Mortality rates assessed 24 h after an acute
697 cold stress (-3.5 °C) for various durations: 90, 120, 135 min are shown in figures C, F and I,
698 respectively (*n* = 100). The black part of the bars represents the percent mortality and grey
699 part is percent survival.



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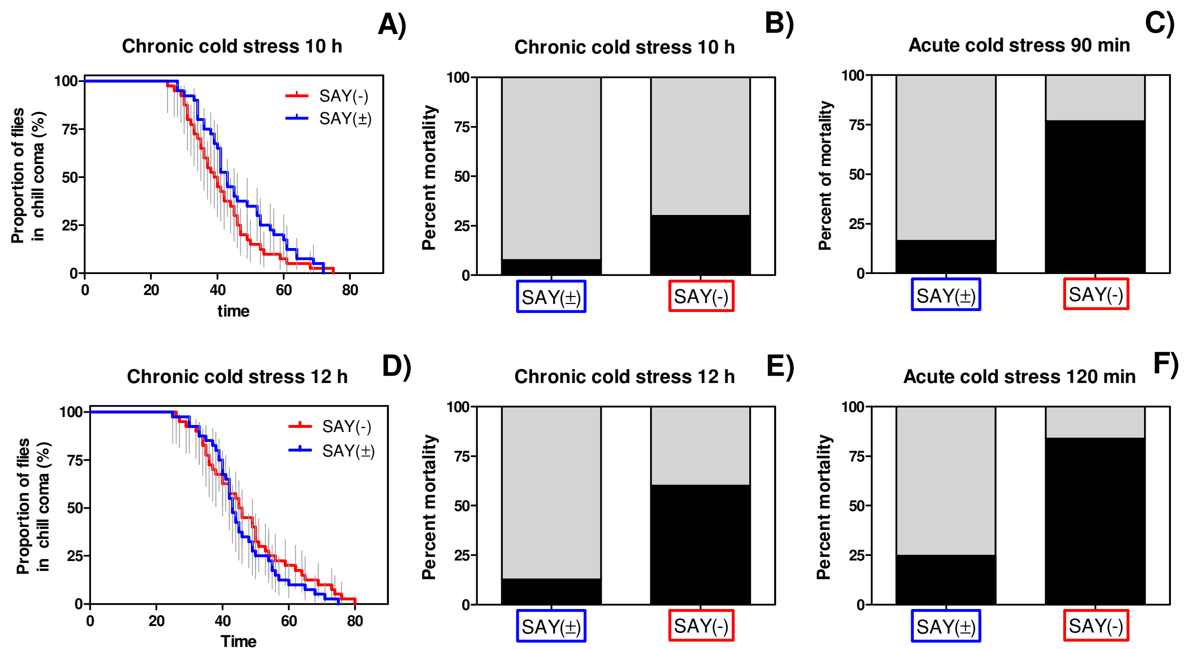
706 **Figure 4:** Composite panel summarizing the cold tolerance assays of the experiment 2.
707 Temporal recovery curves of live yeast-fed females [blue line, SAY(±)] and live yeast-
708 deprived females [red line, SAY(-)] exposed to chronic cold stress (0 °C) for various
709 durations: 10 and 12 h in figures A and D, respectively. Each line represents the mean
710 proportion (± 95% confidence interval) of recovering flies in relation to time after cold stress
711 (n = 50). Mortality rates, assessed 24 h after the chronic cold stresses, are shown in figures B,
712 and E for each nutritional treatment [SAY(-) *vs.* SAY(±)] (n = 50). Mortality rates assessed 24
713 h after an acute cold stress (-3.5 °C) for various durations: 90 and 120 min are shown in
714 figures C and F, respectively (n = 100). The black part of the bars represents the percent
715 mortality and the grey part is percent survival.



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723 **Figure 5:** (A) Multivariate analysis (PCA) based on the GC/MS metabolomic data of the
724 experiment 1 illustrating the plotting of PC1 against PC2. The unit "d" (top right of the plot)
725 represents the side-length of a square in the grid. A clear separation was observed between
726 live yeast-fed [blue ellipse, SAY(+)] and yeast-deprived metabotypes [red ellipse, SA]. Lines
727 link replicates to their respective centroids (*n* = 6). (B) Correlation values of the different
728 metabolite concentrations to the principal components PC1 in the principal component
729 analysis. Correlations are ranked on Y-axis according to their values. Blue bars for negative
730 correlations (*i.e.* accumulated in SAY(+) flies) and red bars for positive correlations (*i.e.*
731 accumulated in SA flies). See Dataset S1 for detailed concentrations and fold changes.

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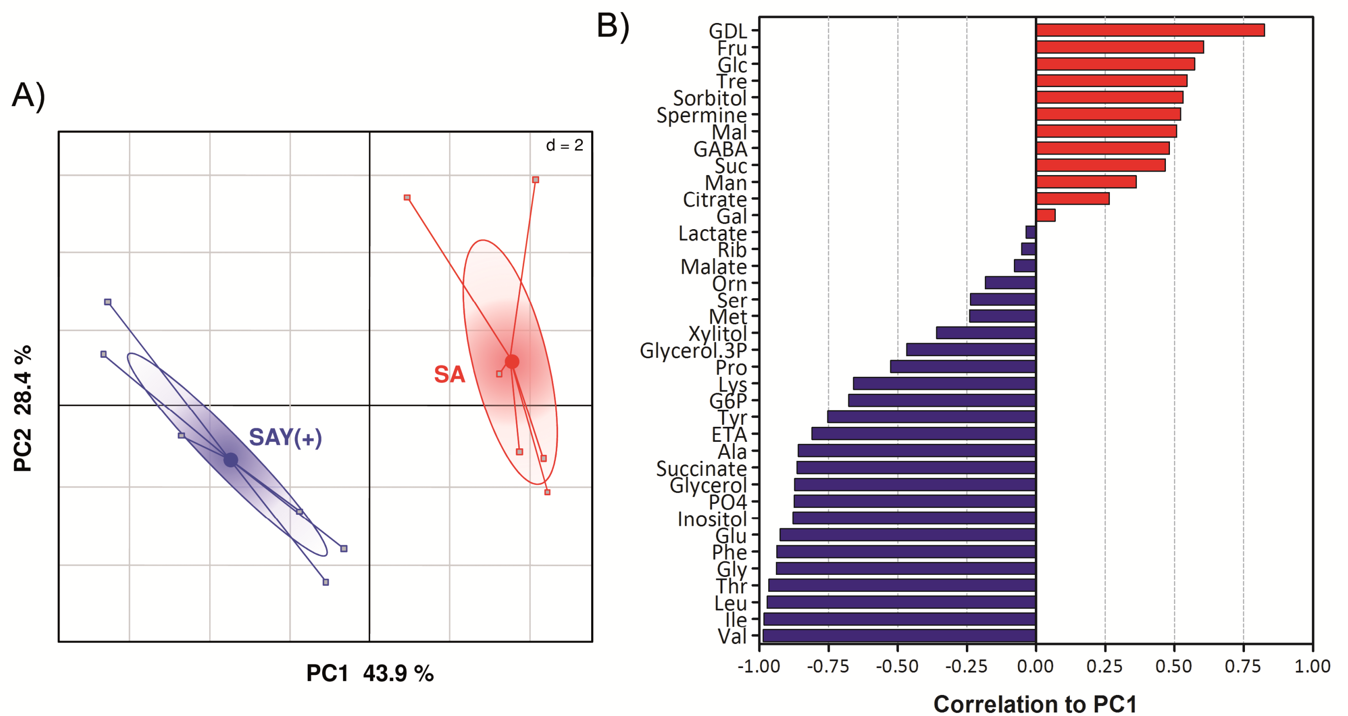

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740 **Figure 6:** (A) Multivariate analysis (PCA) based on the GC/MS metabolomic data of the
741 experiment 2 illustrating the plotting of PC1 against PC2. The unit "d" (top right of the plot)
742 represents the side-length of a square in the grid. A clear separation was observed between
743 live yeast-fed [blue ellipse, SAY(±)] and live yeast-deprived metabotypes [red ellipse,
744 SAY(-)]. Lines link replicates to their respective centroids (n = 6). (B) Correlation values of
745 the different metabolite concentrations to the principal components PC1 in the principal
746 component analysis. Correlations are ranked on Y-axis according to their values. Blue bars for
747 negative correlations (*i.e.* accumulated in SAY(±) flies) and red bars for positive correlations
748 (*i.e.* accumulated in SAY(-) flies). See Dataset S2 for detailed concentrations and fold
749 changes.

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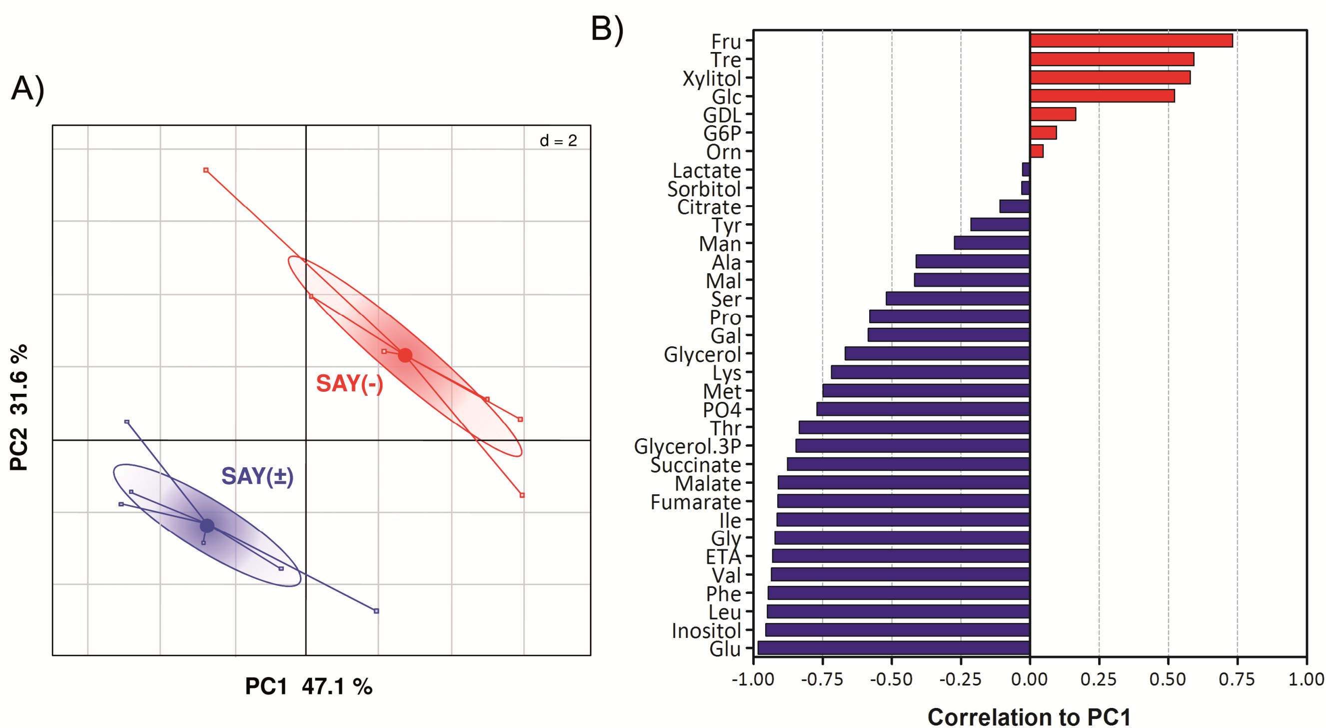

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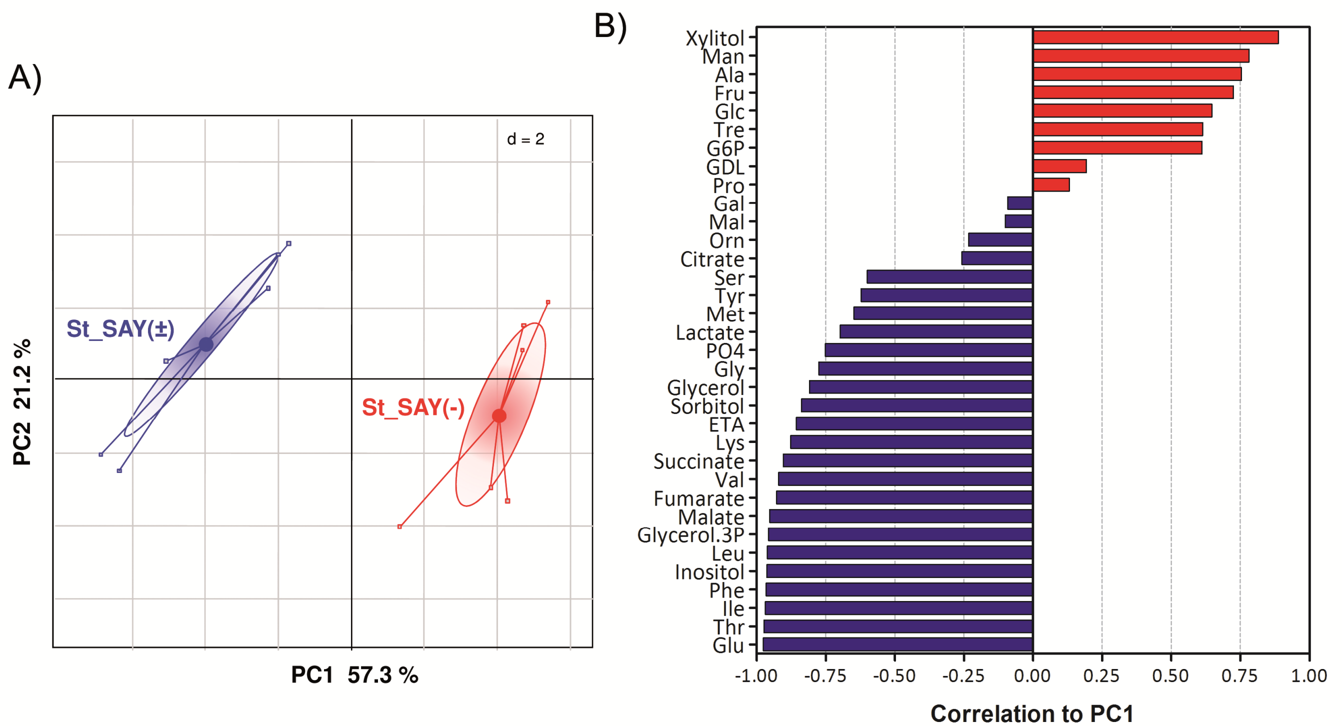
757 **Figure 7:** (A) Multivariate analysis (PCA) based on the GC/MS metabolomic data of the
758 experiment 3 illustrating the plotting of PC1 against PC2. The unit "d" (top right of the plot)
759 represents the side-length of a square in the grid. A clear separation was observed between
760 live yeast-fed [blue ellipse, St-SAY(±)] and live yeast-deprived metabotypes [red ellipse, St-
761 SAY(-)]. Lines link replicates to their respective centroids (*n* = 6). (B) Correlation values of
762 the different metabolite concentrations to the principal components PC1 in the principal
763 component analysis. Correlations are ranked on Y-axis according to their values. Blue bars for
764 negative correlations (*i.e.* accumulated in St-SAY(±) flies) and red bars for positive
765 correlations (*i.e.* accumulated in St-SAY(-) flies). See Dataset S3 for detailed concentrations
766 and fold changes.



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