CHANGES IN THE CONTENT OF SUGARS AND ORGANIC ACIDS DURING RIPENING OF *COFFEA ARABICA* AND *COFFEA CANEPHORA* FRUITS

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Changes in concentration (% of dry weight) and content (mg/organ) of sucrose, glucose, fructose and some organic acids in pericarps and seeds of *Coffea arabica* cv. Mokka, *C. arabica* cv. Catimor and *C. canephora* were monitored during the development and ripening of fruits. The coffee fruits were categorized into five stages 1 to 5 which corresponded to small, medium and large sizes of green fruits, ripening (pink) fruits and fully-ripened (red) fruits, respectively. In all samples, the major sugars in young fruits (stage 1) were fructose (~5 % dry weight) and glucose (~3 % dry weight). Significant amounts of sucrose were also found in the later stages of development. The concentration of sucrose in ripened pericarp and seeds (stage 5) in two cultivars of *C. arabica* (19–25 % of dry weight in pericarp and 8–18% in seeds) was higher than those in *C. canephora* (8 % in pericarp and 5 % in seeds). Sucrose was accumulated exclusively in seeds of two cultivars of *C. arabica*. In contrast, both sucrose and the reducing sugars, fructose and glucose, accumulated in pericarps of all coffee samples and in seeds of *C. canephora*. The concentration of malic acid, citric acid, lactic acid, oxalic acid and quinic acid changed during development and ripening of fruits. The values of most organic acid fluctuated between 0–1%, except for a transient, high content of quinic acid (>2%) in young fruits. Characteristic accumulation patterns of organic acids were found in different organs; citric acid concentration was high in seeds, but malic acid or oxalic acid was high in pericarps. Possible metabolic routes of metabolites are discussed.

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Introduction

Coffea arabica and *C. canephora*, belonging to the Rubiaceae family, are woody, perennial, evergreen dicotyledonous species that are cultivated to produce, respectively, Arabica and Robusta coffee beans. Coffee seeds accumulate sizable amounts of caffeine, trigonelline, chlorogenic acids and sucrose.[1,2] Therefore, the biochemistry and metabolism of coffee plants are interesting from the viewpoint of the diversity of nitrogen and carbohydrate metabolism. In addition, these coffee seed constituents seem to be closely related to the quality of commercial coffee beans.[1] Caffeine and chlorogenic acids increase bitterness, while sucrose and trigonelline are converted to aroma compounds.[3] It has been generally believed that the higher cup quality of *C. arabica* coffee compared to *C. canephora* may be depended on the higher content of trigonelline and sucrose and low levels of caffeine and chlorogenic acids.[4] As a result, concentration of these compounds in seeds before and after roasting were often surveyed by food chemists.[5-8]

As part of a series of our research to elucidate the metabolism in coffee plants, we have reported the fluctuation of the levels of caffeine, trigonelline and chlorogenic acids in pericarps and seeds during growing and ripening fruits of *C. arabica* and *C. canephora*.[9,10] In the present study, we determined the levels of sugars and organic acids in fruits of *C. arabica*, cv. Mokka and cv. Catimor and *C. canephora*. Rogers et al.[11] reported the changes in the levels of various metabolites including sugars and organic acids in developing coffee seeds, but the comparison of metabolites in seeds and pericarps have not been carried out. From the results obtained, we discuss the accumulation profile of sugars and organic acids and possible metabolic pathways related to the biosynthesis of these metabolites.

Experimental

Plant mMaterial

Fruits of *Coffee arabica* cv. Mokka (MA2-7) and cv. Catimor (T5175-7-1), and *Coffea canephora* (#6621), were harvested at the Experimental Station of Hawaii Agriculture Center, Kunia Station, Oahu Island, Hawaii in 2003. These coffee trees were cultivated at the same site at an altitude of ~150 m above sea level. Initially, we collected coffee fruits at the defined dates after flowering using the same branches of the same trees. However, growth of individual fruits varies widely. We therefore defined the growth stages from the sizes and colour of the fruits. In the present study, fruits were divided into five stages according to the growth and maturity of coffee fruits. The final sizes of fruits differed between three plant materials; *C. arabica* cv. Catimor fruits (~1900 mg dry weight) were larger than *C. arabica* cv. Mokka (~500 mg dry weight); *C. canephora* fruits (~480 mg dry weight) were similar but slightly smaller than Mokka.

Analysis of endogenous sugars and organic acids

After harvesting, whole fruits (stage 1) or pericarps and seeds of fruits (stages 2-5) were immediately frozen and lyophilized, and ground to a fine powder using an IKA

Universalmühle M20 blade grinder (IKA-Labortechnik, Staufen, Germany). A powdered sample (~1 g) was mixed with 10 ml of 50 % (v/v) methanol containing 8 mM sulphuric acid. The sample was sonicated for 15 min and then filtered through a glass microfiber filter (Whatman, Grade GF/A), and a 5 ml aliquot of filtrate was evaporated to a volume of less than 2.5 ml using a rotary evaporator. Distilled water was added to make a final volume of 5 ml. The extract (2 ml) was passed through a 1.0 ml bed volume Sep-Pak C18 SPE cartridge (Waters), preconditioned with 1 ml of methanol. Residual unbound extract was eluted by addition of 1 ml of distilled water. The eluent was then filtered through a 0.45 μm nylon filter (Millipore), prior to HPLC.

For HPLC analysis, a Shimadzu SCL-10A controller and LC-10AS pump (Shimadzu Corporation, Japan) were used. Chromatographic separations were achieved using a Rezex ROA organic acid ion exchange column (300 x 7.5 mm) with a Carbo-H^+ guard column (4 mm x 3 mm), (Phenomenex USA, Torrance, CA). The mobile phase consisted of 6.5 mM sulphuric acid in distilled water at a flow rate of 0.4 ml/min. The 24 min run time was followed by a column wash step using a mobile phase of distilled water at a flow rate of 0.52 ml/min for 10 min. The column was re-equilibrated using the original mobile phase at a flow rate of 0.52 ml/min for further 27 min. Sugars and organic acid was detected using a Waters 410 differential refractometer (Waters Associates) and a Shimadzu SPD-10A UV-VIS detector.

Results and discussion

Growth stages of coffee fruits

As mentioned in a previous paper,[12] we have defined the five growth stages of coffee fruits from the sizes and maturation. In the initial stage of coffee fruit development, the fruits consist of pericarp and perisperm. In the later stage, the locular space of seeds is progressively filled with endosperm, up to full seed maturity.[13,14] Whole fruits were therefore used in the stage 1, whereas in the later stages, pericarp and seeds were separated and used in the estimation of caffeine and the content of metabolites.

Fruits of stages 1 to 3 are green-coloured small, medium and large sizes. They corresponded approximately to the rapid expansion and pericarp growth stage, the endosperm formation stage and the dry matter accumulation stage as described by Cannell.[15] Fruits of stage 4, contained pre-ripened seeds, and the fruit skin colour had changed from green to pink. Fruits at stage 5 were fully ripened and red coloured.

Changes in sugar contents

In *Coffea* fruits, sucrose, glucose and fructose are major free sugars. Changes in the concentration of these sugars are expressed as a percent of dry weight and shown in Table 1. Small amounts of glucose and fructose were found in whole fruits at stage 1 and pericarps and seeds at stage 2, but little or no amount of sucrose was detected in any of the samples. In green-coloured large size fruits (stage 3), accumulation of sucrose began in seeds (Figure 1). The concentration in *C. arabica* (6.8% and 4.3% for cv. Mokka and cv. Catimor, respectively) were higher than in *C. canephora* (1.3%). During ripening of fruits, the sugar contents increased both in pericarp and seeds (Figure 1). In pericarps of all the *Coffea* fruit examined, fructose and glucose had accumulated accompanied by an increase in sucrose. In contrast, the major sugar in seeds of *C. arabica* cv. Mokka and cv. Catimor was sucrose (9.9 % and 8.4 % of dry weight, respectively) which, respectively comprised 73 % and 67 % of the total sugars of stage 5 seeds. In contrast, sucrose concentration in *C. canephora* (4.6 % of dry weight) in stage 5 seeds was lower at 27 % of total sugar content (Table 1). In ripened pericarp, total sugar contents in two varieties of *C. arabica* comprised more than 50 % of the dry weight, while the level in *C. canephora* was 26 % of dry weight.

Table 1. Concentration of sucrose (Suc), glucose (Glu) and fructose (Fru) in pericarps and seeds of coffee fruits.

	Stage	Pericarp			Seed		
		Suc	Glu	Fruct	Suc	Glu	Fru
C. arabica cv. Mokka	1*	0.00	3.11	4.98			
	2	0.00	0.57	1.22	0.00	1.62	1.89
	3	1.10	1.47	2.57	6.80	0.00	0.44
	4	12.03	10.42	13.53	10.35	0.88	1.56
	5	19.33	13.47	17.35	9.89	1.54	2.18
C. arabica cv. Catimor	1*	0.00	2.87	4.55			
	2	0.36	0	0.03	0.20	0.00	0.76
	3	1.20	3.98	3.91	4.30	0.78	1.73
	4	10.50	6.51	9.22	4.99	0.62	1.08
	5	24.48	11.94	16.36	8.44	1.62	2.46
C. canephora	1*	0.00	3.06	4.93			
	2	0.00	0.08	0.56	0	0.28	0.84
	3	0.00	0.48	2.03	1.31	0.39	1.23
	4	2.65	2.46	6.61	2.18	0.00	0.80
	5	8.08	5.34	12.63	4.56	5.08	7.18

Values are shown as % of dry weight. *Whole seeds were used in stage 1 and shown in the pericarp column.

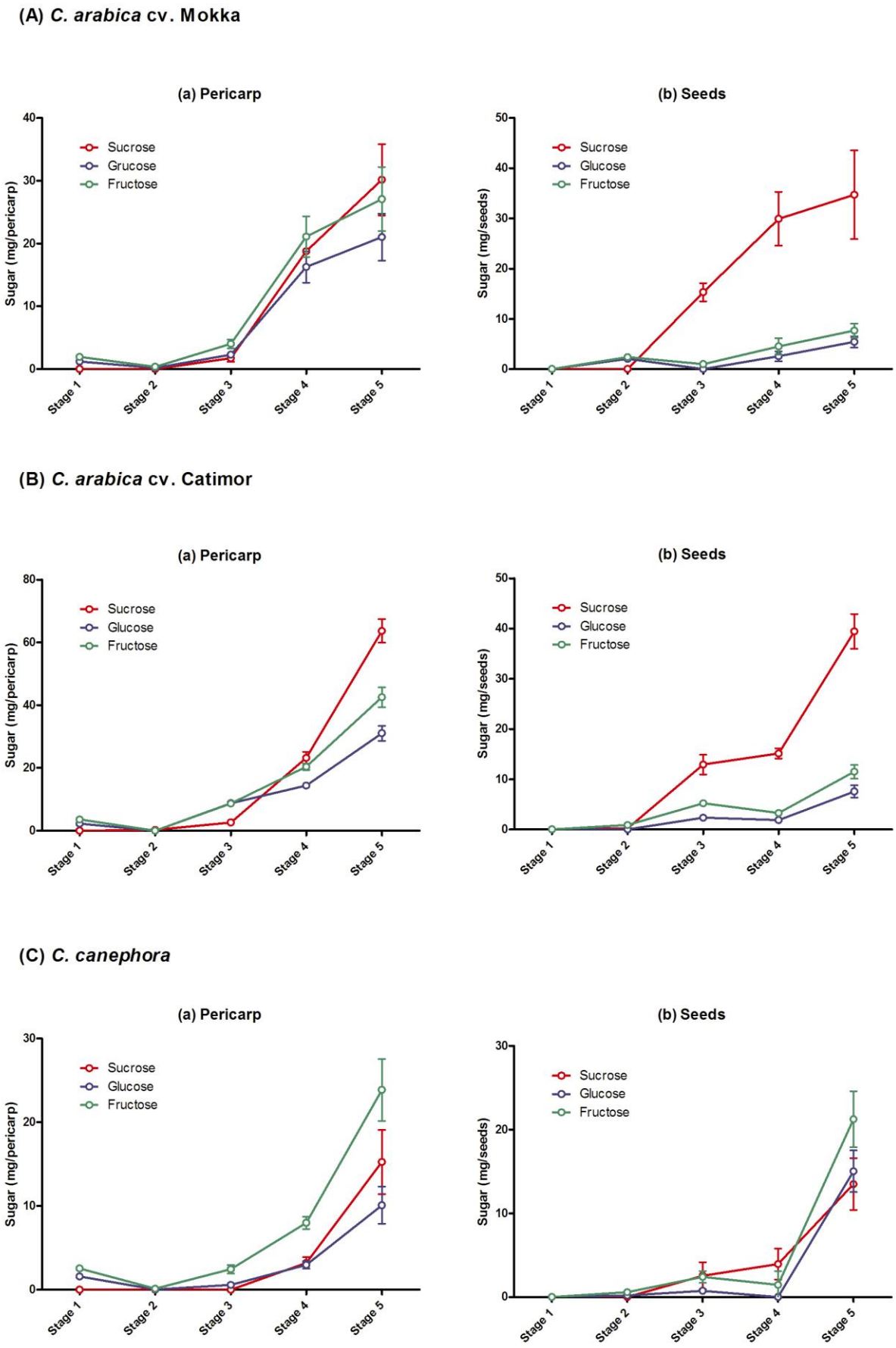

Figure 1. Accumulation of sucrose, glucose and fructose in a pericarp (a) and seeds (b) of *Coffea arabica* cv. Mokka (A), *C. arabica* cv. Catimor (B) and *C. canephora* (C) fruits. A fruit contained two seeds; therefore, the values are expressed as mg per a pericarp (a) or mg per two seeds (b) and SD whole fruits were used in stage 1 and shown the pericarp figures.

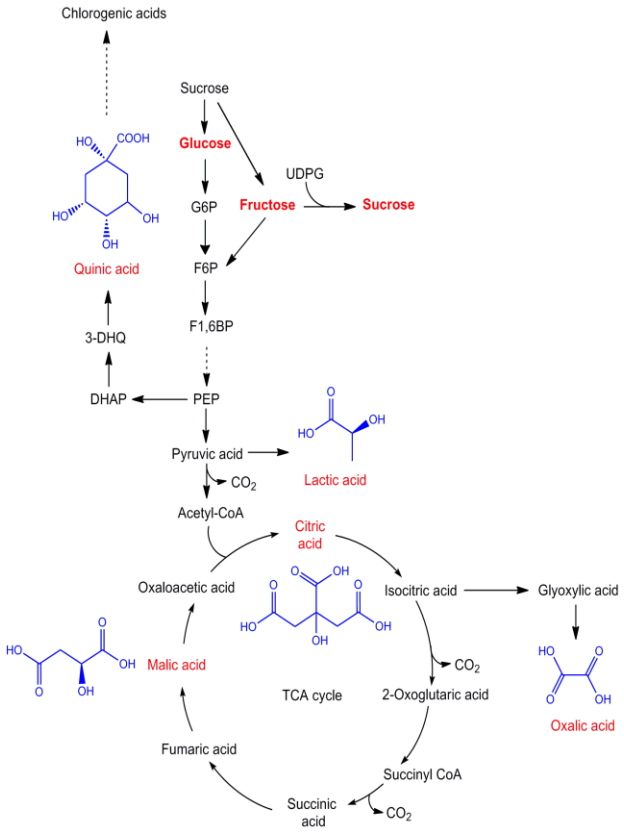

Figure 2. Structures and possible biosynthetic pathways of major organic acids in coffee fruits. DHAP, 3-deoxy-D-arabino-heptulosonic acid 7-phosphate; 3-DHQ, 3-dehydroquinic acid; F1,6-BP, fructose-1,6-bisphosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; PEP, phosphoenolpyruvate; UDPG, UDP-glucose.

Rogers et al.[11] also reported that the levels of glucose and fructose were higher than sucrose in young coffee seeds, but at the end of seed development, sucrose became the major free sugar as a consequence of decrease in the two reducing sugars.

Although the varieties of coffee plants in their research were different from our studies, the sucrose content in *C. arabica* (5–12% of dry weight) was higher than that of *C. canephora* (4–5% of dry weight). Therefore, a higher concentration of sucrose in *C. arabica* compared in *C. canephora* seems to be an inherent character.

Changes in organic acid contents

The present study revealed that the major organic acids in coffee fruits are quinic acid, malic acid, citric acid, lactic acid and oxalic acid. Structures of these organic acids and possible biosynthetic routes in coffee fruits are illustrated in Figure 2. Quinic acid is synthesized from 3-dehydroquinic acid, an intermediate of the shikimic acid pathway.[16] Malic acid and citric acid are members of the tricarboxylic acid cycle (TCA) cycle and lactic acid is formed from pyruvic acid, the end product of glycolysis.[17]

Three pathways for oxalic acid biosynthesis have been proposed in plants, oxidation of glyoxylic acid, catabolism of ascorbic acid and oxaloacetic acid breakdown. Although no research has been published on oxalic acid biosynthesis in coffee plants, a recent study using transgenic rice plants revealed that glyoxylic acid rather than ascorbic acid or oxaloacetic acid is the principal precursor for oxalate biosynthesis.[18]

Quinic acid accumulated in pericarps during the first three stages of development of *C. arabica* and *C. canephora* fruit and then decreased during maturation (Figure 3). The maximum concentrations of quinic acid in pericarps were found in stage 2 of *C. arabica*, cv. Catimor (2.5% of dry weight) and C. canephora (1.6%) and in stage 3 of *C. arabica* cv. Mokka (0.9%), while the concentrations declined in stage 5 of all samples to 0.4–0.5% of dry weight (Table 2). In seeds of all coffee samples, the highest quinic acid concentration occurred at stage 2 (0.5–2.0% of dry weight) and lower values (0.2–0.3 %) were found in stage 5.

Table 2. Concentration of organic acids in pericarps and seeds of coffee fruits.

	Stage	Pericarp					Seed				
		Qui	Mal	Cit	Lac	Oxa	Qui	Mal	Cit	Lac	Oxa
C. arabica cv. Mokka	1*	1.076	0.166	0.234	0.210	0.520					
	2	0.464	0.182	0.164	0.389	0.220	0.500	0.190	0.253	0.412	0.501
	3	0.930	0.605	0.270	0.558	0.809	0.240	0.334	0.689	0.191	0.096
	4	0.679	1.102	0.170	0.601	0.970	0.331	0.528	0.822	0.205	0.156
	5	0.437	0.932	0.276	0.456	1.070	0.270	0.419	0.618	0.171	0.198
C. arabica cv. Catimor	1*	0.550	0.076	0.032	0.186	0.270					
	2	2.460	0.909	0.984	1.665	1.414	0.704	0.425	0.287	0.896	0.502
	3	1.331	0.799	0.336	0.927	0.391	0.344	0.391	0.414	0.37	0.192
	4	0.810	0.829	0.184	0.625	0.355	0.196	0.326	0.489	0.247	0.168
	5	0.383	0.765	0.239	0.375	0.266	0.190	0.332	0.412	0.194	0.141
C. canephora	1*	0.633	0.000	0.071	0.358	0.343					
	2	1.577	0.223	0.229	1.012	0.570	2.045	0.000	0.034	0.151	0.187
	3	1.483	0.253	0.776	1.483	0.967	0.428	0.168	0.244	0.289	0.216
	4	1.006	0.586	0.000	1.566	0.716	0.174	0.331	0.321	0.274	0.165
	5	0.493	0.742	0.323	0.907	0.633	0.183	0.314	0.511	0.306	0.223

Values are shown as % of dry weight. *Whole seeds were used in stage 1 and shown in the pericarp column. Qui, quinic acid; Mal, malic acid; Cit, citric acid; Lac, lactic acid; Oxa, oxalic acid.

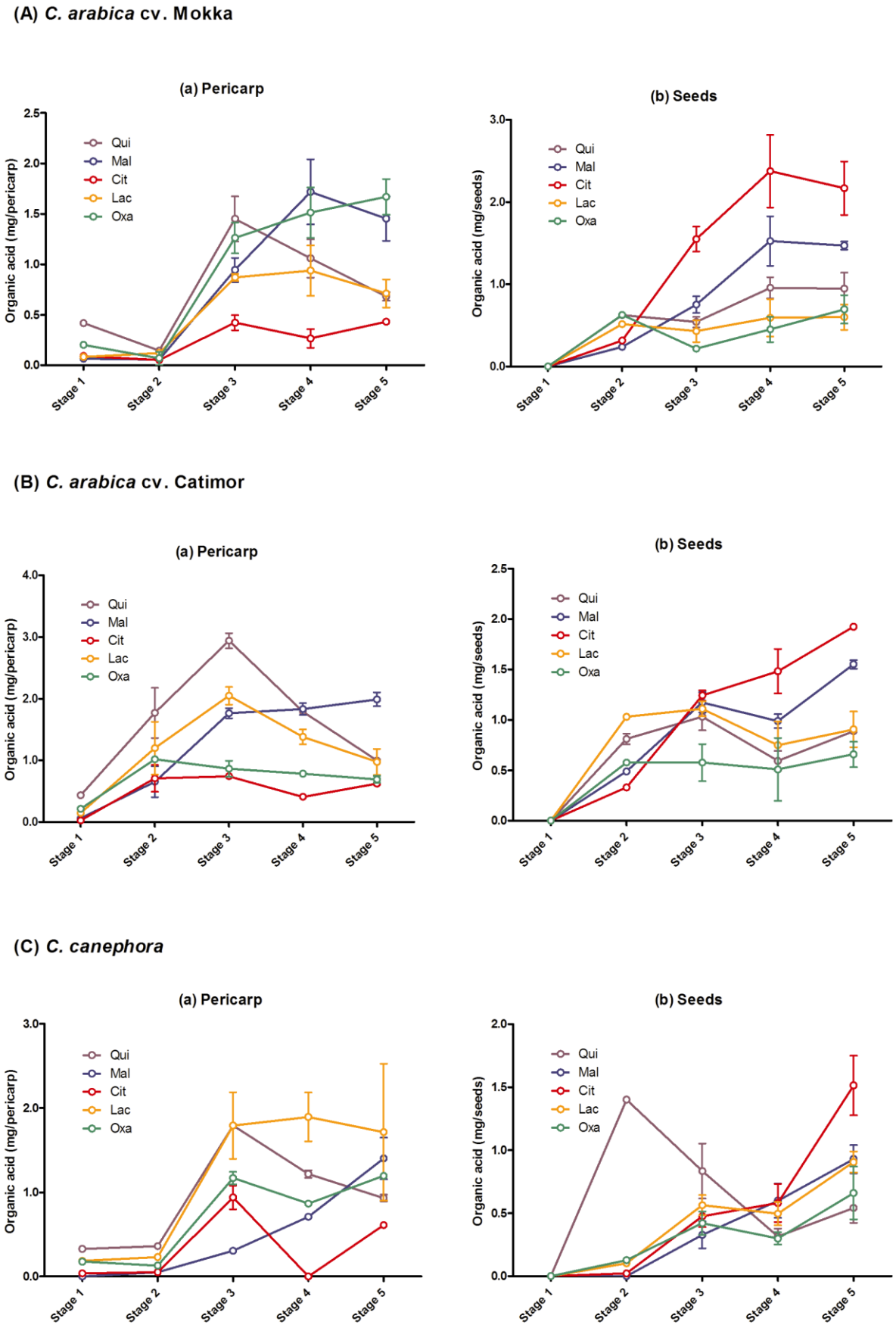

Figure 3. Fluctuation of levels of quinic acid (Qui), malic acid (Mal), citric acid (Cit), lactic acid (Lac) and oxalic acid (Oxa) in a pericarp (a) and seeds (b) during development and ripening of *Coffea arabica* cv. Mokka (A), *C. arabica* cv. Catimor (B) and *C. canephora* (C) fruits. The values are expressed as mg per a pericarp (a) or mg per two seeds (b) and SD whole fruits were used in stage 1 and shown the pericarp figures.

Rogers et al.[11] also reported that high concentration of quinic acid in young coffee seeds and the levels decreased to much lower levels in the end of seed development. Since quinic acid is a polyol moiety of chlorogenic acids, its transient accumulation may be related to its utilization for the synthesis of chlorogenic acids.[10]

The pattern of accumulation of carboxylic acids in pericarp and seeds was different; the most significant difference was found in the accumulation of citric acid which is a major carboxylic acid in seeds (Fig. 3). Concentrations of citric acid in the stage 5 seeds were 0.6 %, 0.4 % and 0.5 % of dry weight of *C. arabica*, cv. Mokka, *C. arabica*, cv. Catimor and *C. canephora*, respectively. In contrast, concentrations of malic acid (0.7–0.9% of dry weight) were higher than those of citric acid (0.2–0.3% of dry weight) in pericarps of all coffee fruit samples (Table 2). Substantial quantities of lactic acid were found in pericarps (0.2–1.7% of dry weight) and seeds (0.2–0.9% of dry weight) in all coffee samples examined (Table 2). Oxalic acid content in pericarp (0.2–1.4% dry weight) is usually higher than in seeds (0.1–0.5% dry weight). Accumulation of oxalic acid was found in pericarps of *C. arabica* cv. Mokka (Figure. 3).

Conclusion

In fruits of coffee plants, sugars and organic acids are synthesized from photosynthates, mainly sucrose, transported from leaves or the epidermal tissues of fruits. Although the detailed metabolic and enzymatic studies have not been investigated in coffee, sucrose transported to sink tissues seems to be degraded to glucose and fructose by invertase and/or UDP-glucose and fructose by sucrose synthase.[19] In *C. arabica*, it has been shown that activity of invertase is higher than that of sucrose synthase in early stages of fruits development.[20] In the growing and developing stages of coffee fruits, hexoses are actively metabolized and organic acids appeared to be synthesized as shown in the routes illustrated in Figure 2.[12] During maturation and ripening stage of coffee fruits, storage of sucrose was initially accompanied by the reduction of metabolic activity,[12] Geromel et al.[20] reported the contribution of sucrose synthase, but not sucrose phosphate synthetase, is important for sucrose accumulation in *C. arabica* seeds at this stage of development. Turnover of carboxylic acids appears to be rapid during fruit development stages where active respiration occurs.[12] In the later stages of fruit ripening, metabolic activity declines and these organic acids may be accumulated as storage compounds. Seed specific accumulation of sucrose and citric acid was observed in both *C. arabica* and *C. anephora*. Arguably, this may be caused by special metabolism in seeds and/or the specific transport of sucrose and citric acid from pericarp to seeds.

The data obtained in this study are important to understand the primary and secondary metabolism of coffee plants. In addition these data are of value when considering metabolic engineering to produce higher quality coffee beans. Since the pericarp of coffee, especially *C. arabica*, contains high concentrations of sugars and organic acids, this tissue also has commercial value which may be used for beverage production.

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