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Short communication

Determination of oil contents in Sacha inchi (*Plukenetia volubilis*) seeds at different developmental stages by two methods: Soxhlet extraction and time-domain nuclear magnetic resonance

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ABSTRACT

Sacha inchi (*Plukenetia volubilis*) seeds are rich in oil that contains a high content of polyunsaturated fatty acids. For genetic improvement and breeding of Sacha inchi, a rapid and nondestructive method for determination of oil content in seeds is critical. In this study, time-domain nuclear magnetic resonance (TD-NMR) was employed to determine oil content in Sacha inchi seeds at different developmental stages, in comparison with the standard Soxhlet extraction method. A highly significant correlation ($R = 0.988$) between oil content determined by TD-NMR analysis and that measured by Soxhlet extraction was found. Compared with Soxhlet extraction, the TD-NMR analysis provides a more accurate determination of oil content in Sacha inchi seeds at the late period of oil accumulation. The results demonstrated that the TD-NMR analysis is a non-destructive and efficient method for reliable determination of oil content in Sacha inchi seeds at different developmental stages.

1. Introduction

Sacha inchi (*Plukenetia volubilis* L.), also known as Inca peanut or Inca inchi, is a perennial, climbing, woody, oleaginous plant of the Euphorbiaceae family, native to the rain forest of South America (Gillespie, 1993, 2007; Hamaker et al., 1992). Sacha inchi seeds are rich in oil (41–54%) and proteins (25–27%) (Gutiérrez et al., 2011; Hamaker et al., 1992). Sacha inchi oil has a high content (approximately 85% of the total oil content) of polyunsaturated fatty acids (PUFAs), namely alpha-linolenic acid (ALA, C18:3, omega-3) and linoleic acid (LA, C18:2, omega-6), accounting for approximately 47–51% and 34–37%, respectively (Fanali et al., 2011; Guillén et al., 2003). Because PUFAs have beneficial effects on human health by preventing several diseases like arthritis, cancer, coronary heart disease, diabetes, hypertension, attention deficit hyperactivity disorder (ADHD), and inflammatory skin diseases (Gogus and Smith, 2010; Hanssen and Schmitz-Hübsch, 2011), Sacha inchi oil has a great potential for applications in the food and pharmaceutical industries (Chirinos et al., 2013). In addition, Sacha inchi oil is also a good feedstock for making biodiesel (Zaccheria et al., 2009, 2012; Zuleta et al., 2012).

For evaluation of germplasm and breeding of high yielding varieties of Sacha inchi with high oil content, a rapid and nondestructive method for determination of oil content in seeds is critical. In this study, time-domain nuclear magnetic resonance (TD-NMR), which is an internationally recognized analytical tool for measuring the oil content in oilseeds (Fuchs et al., 2013; Horn et al., 2011; Pedersen et al., 2000; Todt et al., 2006), was employed to determine oil content in Sacha inchi seeds at different developmental stages, in comparison with the conventional Soxhlet extraction method (Gutiérrez et al., 2011), which is laborious, time consuming and destructive to the seeds.

2. Materials and methods

2.1. Plant materials

One-year-old plants of Sacha inchi (*Plukenetia volubilis* L.) were grown in a field at Xishuangbanna Tropical Botanical Garden (21°54′ N, 101°46′ E, 580 m in altitude) of the Chinese Academy of Sciences located in Menglun town, Mengla County, Yunnan Province, southwest China. The inflorescences were emasculated

Abbreviations: DAP, days after pollination; PUFA, polyunsaturated fatty acids; TD-NMR, time-domain nuclear magnetic resonance.

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in advance of female flower maturation and then bagged to prevent pollination. When female flowers were mature, they were hand-pollinated and tagged with date. Fifteen days after hand-pollination, developing fruits were collected every 5 days except the last two batches of samples which were collected with an interval of 10 days and 15 days, respectively. At each time point, three replicate samples, each with 5 fruits, were randomly collected.

2.2. Seed oil determination by Soxhlet extraction method

Seed oil content was measured by AOAC method no. 920.85 (AOAC, 1990) with an automatic Soxhlet apparatus (Soxtec 2050, FOSS, Denmark) following the manufacturer's guidelines. The Sacha inchi seeds were ground using a Philips mill and passed through a 16-mesh sieve. The dried seed powder (0.8–2.6 g) was packed in a thimble and the oil was extracted with petroleum ether (boiling point: 60–90 °C) for 1.5 h. Upon completion of the oil extraction, the oil was dried at 105 °C for 5 h to remove residual water and petroleum ether. The oil content of the samples was calculated on the basis of dry weight of the seeds.

2.3. Seed oil determination by time-domain nuclear magnetic resonance (TD-NMR)

TD-NMR determination of seed oil content was carried out with the minispec mq-one Seed Analyzer (Bruker Optik GmbH, Germany), equipped with a sample tube of 40-mm diameter. For oil determination in Sacha inchi seeds at different developmental stages, immature seeds were dried at 65 °C until constant weight (for about 48 h), whereas mature seeds with moisture contents less than 10% were used without drying. A calibration curve was obtained from reference samples of oil extracted by Soxhlet method from mature seeds of Sacha inchi.

2.4. Statistical analysis

Data were analyzed by the Statistical Product and Service Solution version 16.0 software (SPSS Inc., Chicago, IL). Differences among means were determined by oneway ANOVA with Tukey's post hoc tests. Graphics were generated using GraphPad Prism 5 (GraphPad Software, San Diego, CA).

3. Results and discussion

3.1. Development of Sacha inchi fruits and seeds

From pollination to mature, the whole growing period of Sacha inchi fruits takes about 145 days (Fig. 1). Both of Sacha inchi fruits and seeds reach full size at 40 days after pollination (DAP), which is about three times of the size of fruits and seeds at 15 DAP (Fig. 1A, B and E). From 40 DAP to mature, no significant change was found in seed size, but fruit size decreases slightly after 115 DAP (Fig. 1B–D and E). At about 115 DAP, the color of pericarp started changing from green into yellowish brown and the seed coat turned black (Fig. 1A–C). At maturity (145 DAP), both fruits and seeds of Sacha inchi are dark brown (Fig. 1D). Similar to the seed size, the seed fresh weight increased rapidly before 40 DAP, after which, until 115 DAP, only a slight increase in seed fresh weight was found (Fig. 1F). After 115 DAP, the seed fresh weight decreased from 1.75 g (115 DAP) to 1.47 g (145 DAP) (Fig. 1F). In contrast, the seed dry weight showed a continuous increase during whole seed development (Fig. 1F). These observations showed that the development of Sacha inchi seeds can be divided into three major stages: the first stage (S1) of rapid gain in size and fresh weight (before 40 DAP, Fig. 1A, B, E and F); the second stage (S2) of rapid dry weight gain (40–115 DAP, Fig. 1F), where size (Fig. 1B, C and E) and fresh weight (Fig. 1F) are relatively stable; the third stage (S3) of fresh weight loss and slight

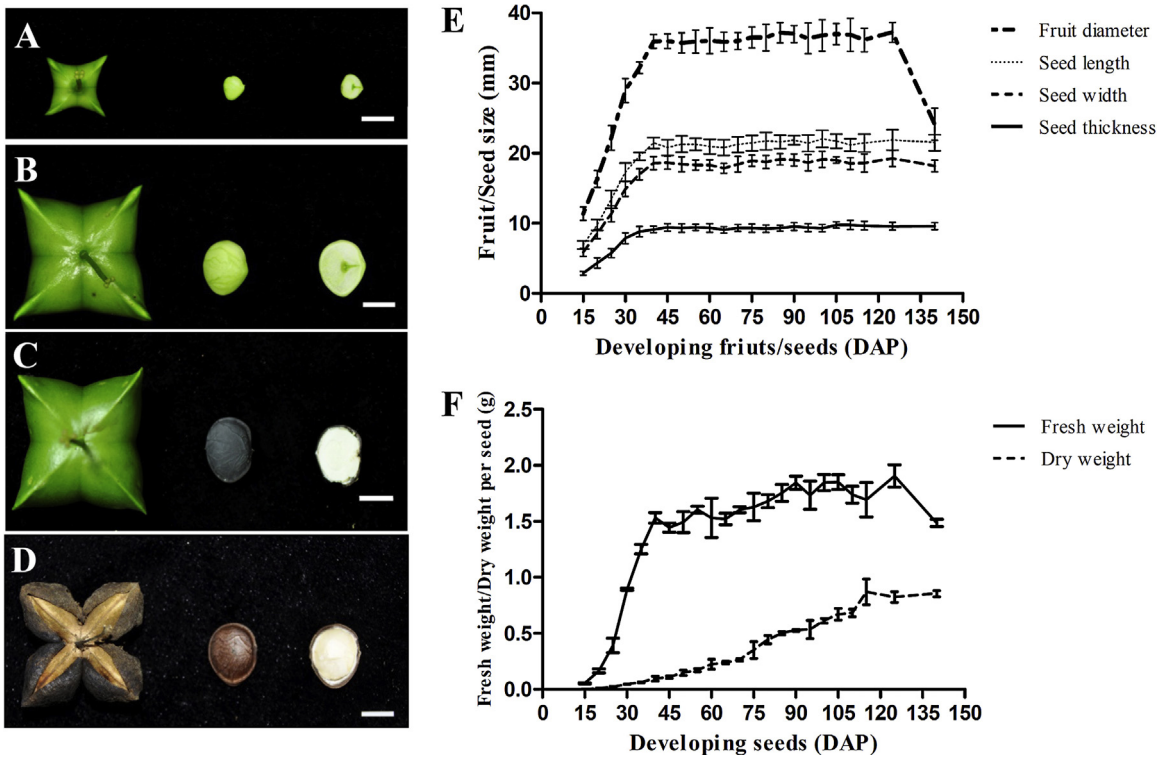

Fig. 1. Developmental changes in morphology, size and weight of Sacha inchi fruits and seeds. (A)–(D) Fruit (left) and intact seed (middle) and longitudinal section of seed (right) at 15 (A), 40 (B), 115 (C) and 145 (D) days after pollination (DAP). Bars = 1 cm. (E) Changes in size of the developing fruits and seeds. (F) Changes in weight of the developing seeds. (E) and (F) Values are means ± standard deviations. At each time point, three replicate samples, each with 5 fruits or seeds, were tested. DAP, days after pollination.

dry weight gain (after 115 DAP, Fig. 1F) as the seed reaches full maturity (Fig. 1C and D). These three stages are of characteristic of seed development of most plants (Greenwood and Bewley, 1982).

3.2. Determination of oil content in Sacha inchi seeds by TD-NMR and Soxhlet extraction

To determine if TD-NMR analysis could be used to quantitate oil in Sacha inchi seeds, oil content in the same batch of seed samples collected at different developmental stages was measured separately by TD-NMR and Soxhlet extraction. The pure oil extracted by Soxhlet method from mature seeds of Sacha inchi was used to calibrate the NMR equipment. Fig. 2A shows the relationship between oil content determined by TD-NMR analysis and Soxhlet extraction. The regression analysis indicated a highly significant correlation ($R = 0.988$) between oil content determined by TD-NMR analysis and that measured by Soxhlet extraction. This result suggests that the TD-NMR analysis is a reliable method comparable to the traditional Soxhlet extraction for determination of oil content in Sacha inchi seeds.

Fig. 2B shows that during the early period of oil accumulation (55–105 DAP), TD-NMR analysis revealed similar seed oil content as that determined by Soxhlet extraction. During the late period of oil accumulation (105–145 DAP), oil content revealed by TD-NMR analysis was slightly higher than that determined by Soxhlet extraction (Fig. 2B). This difference may result from incomplete extraction of lipids in Sacha inchi seeds at late development stage by petroleum ether as found by Finney et al. (1976). In addition, the results of oil determination of mature seeds by Soxhlet extraction with different elution time, shown in Fig. 2C, suggest that a longer elution time (2 h) than that recommended by the supplier of Soxhlet apparatus (0.5–1 h, FOSS, Denmark) is required to extract most of oil from mature seeds. The elution time for extraction experiments described in Fig. 2B is 1.5 h, which is enough for young seeds at early period of oil accumulation (55–105 DAP, Fig. 2B), but insufficient for mature seeds after 105 DAP (Fig. 2B and C).

These results suggest that, in comparison with Soxhlet extraction, the TD-NMR analysis provides a more accurate determination of oil content in Sacha inchi seeds at the late period of oil accumulation (105–145 DAP), from which oil extraction by solvents is more difficult than from young seeds. It is also remarkable that oil accumulation during Sacha inchi seed development (Fig. 2B) shows a similar profile to that of seed dry weight gain (Fig. 1F). This is confirmed by a significant positive correlation ($R = 0.985$) between oil content and dry weight of seeds during seed development (Fig. 2D).

3.3. Conclusions

The results of this study demonstrated that TD-NMR is applicable to determine oil content in Sacha inchi seeds at different

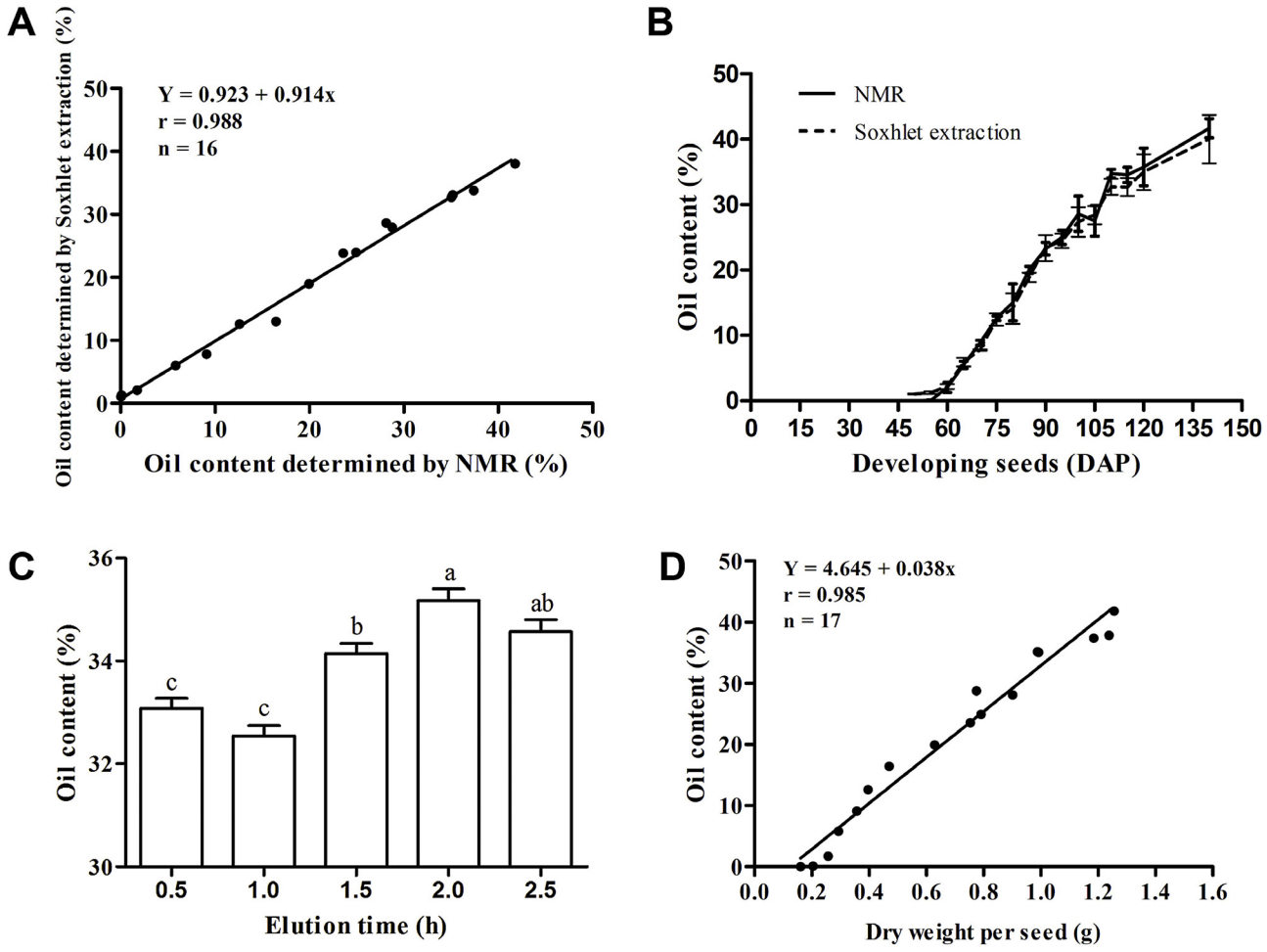

Fig. 2. Determination of oil content of Sacha inchi seeds at various developmental stages by Soxhlet extraction and NMR. (A) Correlation between oil content determined by Soxhlet extraction and by NMR. (B) Oil content of the developing seeds. Values are means ± standard deviations ($n = 3$ extractions or determinations). (C) Effects of elution time on seed oil content determined by Soxhlet extraction. Values are means ± standard deviations ($n = 6$ extractions). Columns with different letters indicate significant differences ($P < 0.05$). (D) Relationship between oil content and dry weight of seeds at various developmental stages.

developmental stages. Compared with the standard Soxhlet extraction, TD-NMR analysis can achieve higher accuracy and efficiency. In addition, since TD-NMR analysis is non-invasive, sample seeds can be measured many times, and used for germination or other analysis after oil determination.

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