Quality control of bullfrog (*Rana catesbeiana Shaw*) oil: a contribution for the extraction and chemical identification

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INTRODUCTION AND OBJECTIVE

The adipose tissue of the bullfrog (*Rana catesbeiana* Shaw) may be used for the extraction of oil, which consists of a complex composition of fatty acids, including essential oil, which are important to maintain homeostasis and normal development of the human organism (Mendez 1998). This oil has been used in popular medicine as a source of essential fatty acids in the prevention of diseases related to the immune system as: asthma, rhinitis, sinusitis and skin diseases (Lopes 2003).

However, the absence of effective parameters to perform the oil extraction and its quality control affect the production and the oil composition. The oil can also be counterfeit with not identified substances or other oils, compromising its (Veiga 2002).

The aim of this work was to perform the physicochemical characterization of bullfrog oil samples from different batches or extraction processes based on indexes values of saponification, acidity, iodine, peroxides and esters. In addition the chemical identification through gas chromatography – mass spectrometry (CG-MS) was carried out.

MATERIALS AND METHODS

Six bullfrog oil samples were gifted from Asmarana Natural Products (Natal, Rio Grande do Norte, Brazil): A (Production Date (PD) : 2009), B (PD: 2010), C (PD: 2011), D (PD: February 2012), E (PD: March 2012), F (PD: April 2012). Additionally, three bullfrog oil samples were extracted from the adipose tissue of bullfrog in the Laboratory of Dispersed Systems (LaSiD) through different methods: sample G (obtained by hot extraction), sample H (obtained by extraction with hexane), and I (obtained by cold extraction). All used reagents were of pharmaceutical grade.

For the extraction of bullfrog oil through hexane, 300g of bullfrog adipose tissue were weighted and crushed in a mixer by 1 minute to generate a crushed extract (CE). Moreover, 10 g of CE and 15 mL of hexane were mixed by Ultra Turrax® for 3 min (13000 RPM), followed by centrifugation at 3000 RPM for 10 minutes and evaporated at 40 °C. The hot extraction was preceded by weighting 10 g of CE subsequently heated at 120 °C for 20 min in silicone bath and centrifuged for 10 min. The cold extraction of CE was performed by pressing and filtrating it with a 0.22 µm pore filter.

Physicochemical and chemical tests were performed according to the USP 35 and the American Oil Chemists Society. For the saponification index (SI), the oil was titrated with 0.5 N hydrochloric acid, using phenolphthalein as an indicator. In the analysis of the acidity index (AI) 0.1 N sodium hydroxide was used to titrate the sample. Concerning the iodine index (II) determination, potassium iodide was used as an indicator and 0.1 N sodium thiosulfate was used to titrate the samples. Moreover, starch was used as an indicator. For the peroxides index, (PI) saturated potassium iodide solution and starch were used as an indicator. Finally, 0.01N sodium thiosulfate was used to titrate the samples. The esters index (EI) was determined by the difference between SI and AI.

To identify the chemical components, the bullfrog oil was analyzed by gas chromatography (Hewlett-Packard 6890) coupled to a mass selective detector HP-5975, using a capillary column HP-5MS (30 m x 0.25 mm x 0.25 µm). The initial column temperature was 110 °C, followed by heating of 5 °C/min to 280 °C (26 min). The detector and injector temperatures were 300 °C and 250 °C, respectively. The volume injected of all samples was 1 µL and the flow of carrier gas (helium super dry) was 1.0 mL/min. The samples were methylated using diazomethane before being injected into the chromatograph.

The identification of compounds was based on the comparison between the mass spectra with the NIST library (Software package, Finnigan). The quantitative determination was based on the retention time and the peaks area.

RESULTS AND DISCUSSION

To ensure the safety and efficacy of natural products, physicochemical controls are sorely needed. The sample A presented the highest AI (Figure1), showing a low state of preservation, which may be accelerated by light exposure, heating, wrong storage and unsatisfactory extraction process. However, the sample F and H showed low AI compared to the sample G, which may indicate that the extraction using solvents provides better stability of the oil (Moretto 1998).

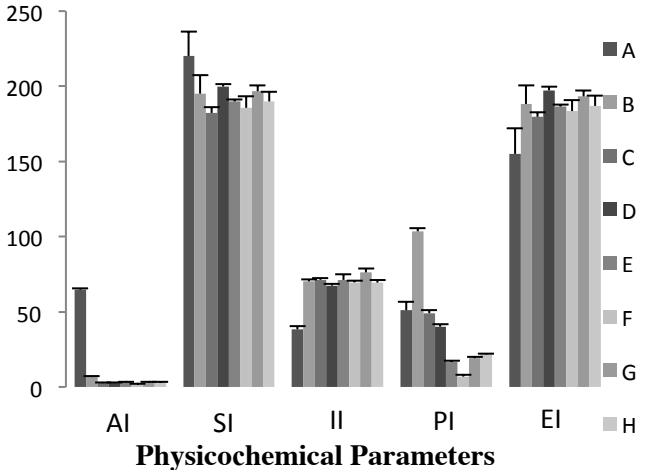

Figure 1: Physicochemical parameters resulting from the analysis of bullfrog oil samples. Samples: A, B, C, D, E, F, G, H. AI (mg of KOH / g of oil); SI (mg of KOH / g of oil); II (g of iodine absorbed / 100 g of oil); PI (mEq of active oxygen / 1000 g of oil); EI (mg of KOH /g of oil).

The II gives the degree of unsaturation of fatty acids in the oil (Knothe 2002). The sample A showed the lowest value for this index, indicating that this sample has a higher saturated fatty acid content. Peroxides are primary products of lipid oxidation (Moretto 1998). Sample B presented the higher PI, indicating a higher degree of oxidation than the sample F that showed the lowest index value. Therefore, the oil with the oldest extraction process may suffer further oxidation. All samples showed high SI. Sample A presented slightly higher SI probably due to the elevated amount of free fatty acids (Moretto 1998). The EI was higher for the sample D indicating a higher amount of triglycerides in the sample.

Palmitic acid was the major content of saturated fatty acids in the oil, followed by stearic acid (Figure 2), corroborating the results of Mendez et al (1998). Concerning the monounsaturated acids, oleic acid showed predominance compared to palmitoleic acid content (Figure 2). For the polyunsaturated fatty acids, linoleic acid was the major compound of this group. Arachidonic acid, which is an essential fatty acid of the omega 6 family, was also identified into the samples.

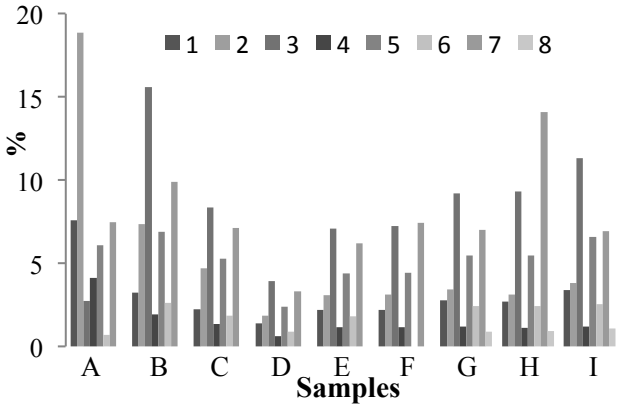

Figure 2: Percentage of the main compounds of the bullfrog oil samples.1: Palmitoleic acid; 2: Palmitic acid; 3: Oleic acid; 4: Stearic acid; 5: Linoleic acid; 6: Glycerol 1,2 dipalmitate; 7: Glycerol 2 monooleate; 8: Arachidonic acid.

CONCLUSIONS

The different values found for: AI, SI, II, PI and EI may indicate that the extraction method and the storage conditions can influence the stability and the oil quality. Additionally, the analysis by GC-MS revealed the presence of expressive amounts of linoleic acid in almost all samples and the presence of arachidonic acid only in samples G, H and I, which were produced in our laboratory. However, the extraction with hexane obtained higher yield, but the cold extraction showed a higher amount of polyunsaturated fatty acids. Thus, it may be concluded that the standardization of the oil extraction and evaluation of their physical chemical control are of great importance to affirm the quality and the pharmacological properties of bullfrog oil.

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