IDENTIFICATION OF PHENOLIC COMPOUNDS IN POMEGRANATE (*Punica granatum*) SEEDS AND SOYBEAN (*Glicine max*) OILS AND ITS STABILIZATION BY SPRAY DRYING

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ABSTRACT – Pomegranate seed oil (PSO), even though highly sensitive to oxidation, has been extensively studied due to its bioactive potentials, assigned to the punicic acid, a polyunsaturated fatty acid, and several phenolic compounds. Combined with soybean oil (SO) we get an affordable product, rich in essential fatty acids. In this study we evaluate PSO and SO for phenolic and fatty acids compositions and encapsulate it by spray drying to increase its shelf life. Modified starch, maltodextrin and gum arabic are evaluated by a mixture design to get the better wall material composition. The content of punicic, a conjugated linolenic acid, and linoleic acids in PSO and SO were, respectively 75% and 54%. As expected, the phenolic acids were found at low concentration in PSO and SO, but in high content in pressing cake. The better response for spray drying process yield and oxidative stability were achieved at using modified starch and gum arabic at the same proportion as wall material.

KEYWORDS: pomegranate seed oil, phenolic acids, fatty acids, microencapsulation.

1. INTRODUCTION

The industry of fruit juices plays a growing part in global market, especially in Brazil. The generated residues can become an environmental problem if not properly disposed. Such residues may contain vegetable oil and several bioactive compounds that, if recovered, can help to prevent diseases and maintain health. The quality and application of vegetable oils largely depends on its saturated and unsaturated fatty acids composition and profile and on its smaller compounds or fraction unsaponifiable. The seeds in pomegranate residue generated from juice extraction contain a special oil rich in polyunsaturated fatty acids and phenolic compounds with high anti-oxidant and anti-inflammatory capacity (Silva, 2013).

Pomegranate seed oil contains more than 70% of punicic acid, an unsaturated fatty acid with 18 carbons and 3 double bonds (C18:3), conjugated isomer of linolenic acid (CLnA), of the ω-3 family (Arao et al., 2004). Reported data (Lucci et al., 2015, Viuda-Martos et al., 2010) have shown that punicic acid presents anti-tumour and anti-inflammatory properties. Besides it contains phenolic compounds that in addition to the punicic acid may be related to the elevated antioxidant capacity

attributed to the oil. In spite of this, punicic acid has not been explored as an ingredient for functional food formulation.

Phenolic acids, a large group inside the phenolic compounds, are largely found in vegetables. They have been extensively studied due to its antioxidants, anti-microbial and anti-cancer properties (Lutterodt et al., 2010). Phenolic acids can be divided into two major groups, hydroxibenzoic and hidroxicinnamic acids. They are more hydrophilic then lipophilic and therefore are less present in vegetable oils. Although the phenolic acids in pomegranate juices are well documented, there are few studies dealing with its identification in pomegranate seed.

Brazil has the second largest soybean production in the world (Embrapa Soja, 2015), and organic pressed soybean oil is stable and contains vitamins E, K, lecithin and a high content of linoleic acid, an essential fatty acid of the ω-6 group. A blend of these oils may provide the recommended nutritional specifications to the ω-6/ ω-3 ratio in human nutrition (Simopoulos, 2002) while making it a more affordable product, capable of reach wider markets and different consumers.

However, the large number of unsaturated chains make pomegranate oil highly reactive and thus, highly sensitive to oxidative rancidity (Silva, 2013). In order to be commercialized, it must be stable and secure. Spray drying is one of the most industrially used processes for microencapsulating process and makes it possible toprotect a sensitive material by creating a physical barrier (shell) between the material (core) and oxidizing agents such as oxygen and light (Kaushik et al., 2014).

Spray drying the oil in the suitable conditions with the appropriate wall material can create stable microcapsules that both protect the oil against rancidity and increase its shelf life. A good wall material protects the core and increases the yield since it increases the glass transition temperature of the product, reducing its stickiness, wall deposition at the drying chamber and the agglomeration tendency of the powder (Oliveira et al., 2010).

The inlet temperature, inlet air flow rate, feed flow rate and the encapsulating material are parameters that control characteristics of the product. For microencapsulation by spray drying a first step of formulating with carriers agents is required for heat protection and facilitate its flow rate into the tower. Encapsulating agents most commonly used are modified or hydrolyzed starch and gum arabic.

2. MATERIALS AND METHODS

Material: Fresh pomegranates (*Punica granatum* L.) fruits were supplied by Boa Fruta Farm, located in Petrolina, Brazilian semiarid region.

Pomegranate oil: Seeds with 10% moisture content were triturated in a knife mill and crushed in continuous hydraulic press to yield a crude oil (Silva et al., 2012). After decanting for separation of impurities, pomegranate seed oil (PSO) obtained was used in the formulation.

Soybean oil: organic soybean (SO) oil obtained by cold pressing (Organic®) was purchased in store specialized in high quality products in local trade).

Fatty acids identification: Total fatty acid esterification followed the Hartmann-Lago method (1973) and the chromatographic analysis was made in chromatograph Agilent Technologies, model 7890A, column DB-WAX (30 m x 250 µm x 0.25 µm) and mass detector Agilent Technologies, model 5975C VL MSD. Temperature was 100 °C for 2 min, followed to 150 °C by 10 °C/min and raising to 180°C by 3 °C/min, standing for 20 minutes. Injector and detector temperatures were adjusted to 210 °C and 200 °C, respectively. Samples were diluted to 1% in heptane and the injected volume was 1 µL with Split 1:100. Helium flow as carrier was 3 mL/min.

Phenolic acids and flavonoids identification: From 1 g of sample, the free phenolics were extracted with 4ml polar and nonpolar solvents (methanol:water 50:50 and acetone:water 70:30 v:v) for 1h at 25 °C each, respectively, and the supernatant were separated by centrifugation (extract 1). The precipitant was then hydrolyzed with 5 mL of NaOH 2 M/10 mM EDTA/ 1% ascorbic acid solution at 62 °C for 1 h. Hydrolysis was then stopped with 1.5 mL of HCl 6 M and it rested to room

temperature. After centrifugation, 6.5 mL of ethyl acetate were added twice to the supernatant, and after agitation on vortex the solution went to ultrasound for 5 min. The organic phases were combined and evaporated in N_2 flow. The residue were resuspended at 2 mL of methanol:water (80:20 v:v), took to ultrasound for 5 min and filtered in 0.45 µm filter membrane through a glass syringe (extract 2). Both extracts were analyzed in HPLC Waters® Alliance model 2690/5 with photodiode array detector Waters® model 2996 (scanning 201-600 with measurement at 270 nm), Empower® software, thermo column HYPERSIL BDS C18 (100x4.6mm, 2.4 µm). Column temperature 30 °C. Detector phosphoric acid gradient elution mode 1.5 $ml.min^{-1}$ in water (phase A) and acetonitrile (phase B) flowing from 1.0 to 1.2 $mL.min^{-1}$, injection volume of 10 µL and run time of 28 minutes.

Oxidative Stability: it was measured by induction time (IT) in Rancimat 743 equipment according to EN 14112 method but at 80 °C and air flow 20 $L.h^{-1}$.

Formulation: Pressed pomegranate seed oil was diluted in organic cold pressed soybean oil at 20%. Modified Starch (MS, Capsul®, Ingredion™), Maltodextrin DE5 (MD, Globe® 1805) and Arabic Gum (GA, Vetec) were used as encapsulating materials in a simplex centroid three level mixture design, as can be seen in Table 1. The proportion selected for oil:wall material was 1:4, and the total ratio of water (62.5%) and oil (7.5%) in the emulsion were fixed for all tests.

Table 1. Parameters of mixed experimental design

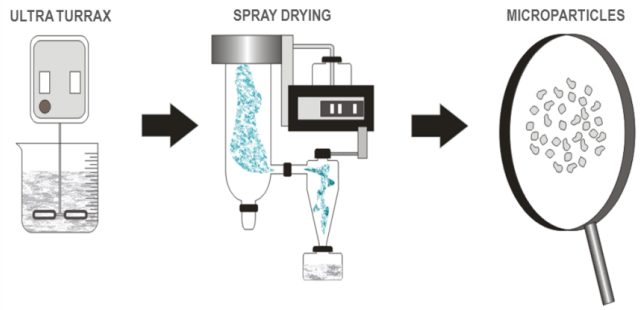
Test	Encapsulating material		
	Maltodextrin (%)	Gum Arabic (%)	Modified Starch (%)
1	30.0	--	--
2	--	30.0	--
3	--	--	30.0
4	15.0	15.0	--
5	15.0	--	15.0
6	--	15.0	15.0
7	10.0	10.0	10.0
8	10.0	10.0	10.0
9	10.0	10.0	10.0

Fixed proportion of water (62.5%) and oil (7.5%).

Data analysis: All the data evaluation was performed in Statistica 10.0 Software.

Spray drying: Figure 1 shows the simplified scheme of the spray drying process. for each test the oil, Encapsulating material and water were mixed to form an emulsion with the ultra turrax, which was led to Buchi B-290 spray dryer at inlet temperature 150 ºC, co-current spray gas flow rate 414 $L*h^{-1}$ and feed flow rate 0.36 $L*h^{-1}$. The powder product was packed in sealed metallic packaging and stored at -18 ºC until the moment of analyses. The yield (Y) was calculated as the ratio of the mass of product (dry basis) and mass of solids in the feed.

Figure 1. Scheme of spray drying



3. RESULTS

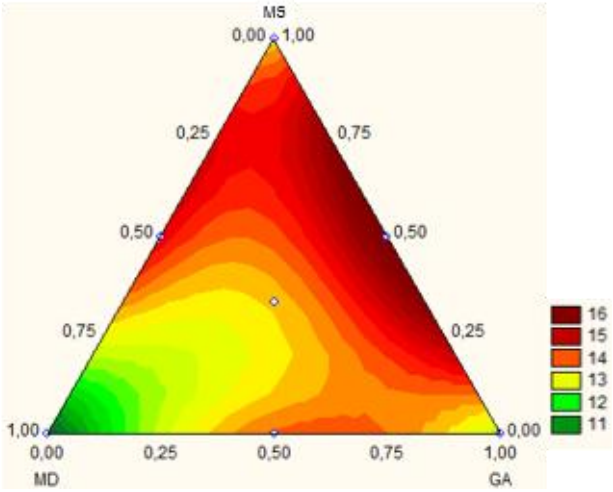
Fatty acids (FA): Punicic acid (C18:3) was the major fatty acid found in pomegranate seed oil (PSO), with 75.5% of total area. The saturated acids palmitic (C16:0), stearic (C18:0) and behenic (C22:0) were found at 3.0, 1.7 and 3.2 % of total area, and the unsaturated oleic (C18:1), linoleic (C18:2) and erucic (C22:1n9) at 4.7, 5.3 and 5.5 % of total area, respectively, were also present. In soybean oil (SO), the main fatty acids were palmitic, stearic, oleic and linoleic with respectively 8.0, 4.4, 23.4 and 54.2 % of total area. Linoleic acid (omega-6) and linolenic acid (omega-3) are essential fatty acids, i.e. cannot be synthetized by the human body and need to be present in the feeding. The relationship between the ratio omega-6/omega-3 have influence in human health. Today's western diets have high levels of omega-6 and are deficient in omega-3 fatty acids, promoving a very high omega-6/omega-3 ratio in human diet. According to Simopoulos (2002), this diet promotes the pathogenesis of many diseases, such as cancer, cardiovascular, inflammatory and autoimmune diseases. Low omega-6/omega-3 ratio, on the other hand, present suppressive effect.

Phenolics acids (PA) and flavonoids: In pressed SO were identified ellagic and siringic acids. In turn, in pressed PSO were identified ellagic and vanilic acids. Nevertheless, in pomegranate pressed cake (PPC) 5 PA (protocatecuic, p-hidroxibenzoic, siringic, ferulic and elagic acids, in crescent order of peak height) were identified before hydrolysis and 7 (the same previously identified plus vanilic and p-cumaric acids) after it. Elagic, siringic, protocatecuic, ferulic, p-hidroxibenzoic and p-cumaric acids showed antioxidant activities against free radicals (Garcia-Niño & Zazueta, 2015, Heleno et al., 2015, Mancuso & Santangelo, 2014). In general, the PA are hydrophilic compounds, and remains, preferentially, in pressed cake. This antioxidant property may be able to protect the oil against oxidative rancidity, increasing its quality and shelf life, discarding the necessity of synthetic preservatives, recently linked to potentially harmful effects. Extractions using polar solvents as ethanol instead of pressing process can transfer these PA to pomegranate oil, making the oil more bioactive and, thus, more valuable.

Microencapsulation: Ranging the input parameters it is possible to control the outlet temperature and product humidity, and the particle diameter. The input air temperature should not be too high if the core is thermo sensitive. On the other hand, if the outlet temperature is too low, the final humidity will be too high, which is not desirable since it will affect the stability of the product. The final humidity of microencapsulated product (pomegranate and soybean oils) was 3.1% and the outlet air temperature was 76 °C. This is the effective temperature achieved by the surface of the particles during the drying, due to thermodynamics equilibrium.

Figure 2 shows the statistical analysis for the dependent variable "Oxidative Stability". The induction time (IT) expresses a time interval until the sample reaches a high oxidation level, according to the method in use at 80 °C and 20 $L.h^{-1}$. For oil mixing the IT was 11.4 h and for the microencapsulated samples the highest IT was found at 16.2 h using MS:GA formulation (test 6), 42% higher. Lutterodt et al (2011) found values of IT among 19.7 and 23.4 h for grape seed oil at 80 ºC and 7 $L.h^{-1}$. Probably, these higher values reported in the literature is due to the lower air flow applied reducing the oxidation rate.

Figure 2. Response surface for Oxidative Stability expressed as induction time (h).



MS- modified starch; MD- maltodextrin; GA- Gum arabic

4. CONCLUSION

Both PSO and SO can be considered as an essential fatty acids source for human diets. Furthermore, PSO and SO have phenolic compounds with antioxidant properties.

Microencapsulation protected the pomegranate oil, increasing the oxidative stability of final powder. The formulation with equal proportions of MS and GA as wall material provided the best results for oxidative stability, producing stable microcapsules.

5. SUGGESTIONS

For future works, the extraction of phenolic acids from the pressed cake, for addition as natural antioxidant in SO and PSO, should be evaluated.

6. ACKNOWLEDGEMENT

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