Adsorption Isotherms of Squash (*Cucurbita moschata*) Seed Oil on Activated Carbon

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With vegetal carbon as adsorbent (5% w/w), the effects of temperature (30°C and 50°C), concentration of H_2O (0% and 25%) and adsorption time (0 hr, 1 hr, 2 hr, 3 hr) on the chemical characteristics of unrefined *Cucurbita moschata* seed oil were studied in a batch adsorption system by using a "split-plot" experimental design. With the exception of the iodine value, the chemical properties of the oil (saponification and peroxide value, carotenoid, and free fatty acids concentration) were affected significantly by interactions among the adsorption time, temperature and concentration of H_2O in the adsorption system. The results suggest that the physicochemical characteristics of the oil, and therefore its functional properties, may be modified and controlled by the conditions utilized during the adsorption process.

KEY WORDS: Activated carbon, adsorption isotherms, bleaching, seed oil.

Crude oils and fats contain varying amounts of substances that may impart an undesirable flavor, color or odor. With the exception of virgin olive oil, unrefined or "crude" oils are subjected to a number of commercial refining processes, both physical and chemical (namely degumming, neutralizing, bleaching and deodorizing), designed to remove these materials (1-3). For example, degumming removes phospholipids and mucilaginous material; alkali refining removes free fatty acid, phospholipid, metals and chlorophyll. The process of bleaching reduces chlorophyll, carotenoid, peroxide and residual fatty acid. Deodorization removes volatile compounds, carotenoid, free fatty acid and tocopherol, and decomposes peroxide, which results in improved flavor quality and stability of the oil (2-4).

The adsorptive process of bleaching, achieved through the use of matrices, such as bentonite, diatomaceous earth and carbon, is mainly intended to improve oil color (5). However, several reports have shown additional effects on the concentration of trace metals, phospholipids (6), peroxides (7,8) as well as in the products of peroxide decomposition (9). Bleaching is the only operation in the refining of vegetable oils that makes use of an adsorption process. Brown and Snyder (6) and Toro-Vazquez (8) have pointed out that studies on the selective adsorption of undesirable components from "crude" oils could lead to an increased efficiency of refining methods, a better understanding of bleaching procedures and a better control of the physicochemical/functional properties of refined oils.

The process of adsorption involves separation of a substance from one phase accompanied by its accumulation or concentration on the surface of the adsorbing phase. An equilibrium distribution between phase concentrations is defined for each combination of specific adsorbate, adsorbent, and set of system conditions (*i.e.*, temperature, pH, ionic strength) (10).

The objective of this paper was to evaluate, in an adsorption system with activated carbon, the effect of adsorption time, temperature, and concentration of H_2O in the adsorption system on the chemical characteristics of squash (*Cucurbita moschata*) seed oil. A "split-plot" experimental design was used in order to improve the precision in the evaluation of the interactions of contact time with temperature and water concentration in the system. Previous research (8) has described the physicochemical and extractive characteristics of this oil.

MATERIALS AND METHODS

Materials. The reagents utilized were analytical grade. The activated carbon was provided by Clarificantes Mexicanos (Santa Clara, Estado de México, México). To eliminate secondary effects, such as particle size and moisture content, the same lot of carbon used in all the experiments was sieved (U.S. standard, −14/+24), washed (agitation for 24 hr in twice the volume of deionized water), filtered and dried (100°C for 24 hr).

Adsorption process. Cucurbita moschata oil was isolated from whole seed obtained from a local market. The whole seeds (2 kg) were milled, dried (100°C for 4 hr) and oil was extracted as follows. A mixture of milled seed and n-hexane (ratio 1:4) was stirred magnetically (30 min) and then filtered (Whatman paper #2). The solvent was evaporated from the filtrate under vacuum at 60°C. In order to homogenize the oil sample, different oil batches were pooled and magnetically stirred for 15 min. The "crude" oil was distributed in aliquots of 200 mL in 250-mL amber bottles and stored under nitrogen atmosphere and refrigeration (4°C).

Adsorption experiments were made with activated carbon as the adsorbent. The effects of adsorption time (0 hr, 1 hr, 2 hr, and 3 hr), temperature (30°C and 50°C), and concentration of H_2O in the adsorption system (through the addition of deionized water, 0% w/w and 25% w/w) on the oil chemical characteristics were studied. The combination of temperature and concentration of H_2O in the system was done in a 2^2 factorial arrangement, distributing the treatments among the oil bottles in a complete randomized experimental design with two replicates. The adsorbent concentration was kept constant (5% w/w) in all the experiments. Previous research (8) has shown that this adsorbent concentration is needed in order to obtain significant reduction in peroxide, free fatty acid and carotenoid concentration. The adsorption process was carried out under vacuum in a rotary evaporator (Büchi RE 120; Büchi Laboratoriums-Technik

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AG, Flawil, Switzerland). The appropriate proportions of oil, adsorbent, and deionized water when applicable, were weighed in the evaporator flask (1 L) to a final weight of 200 g. The mixture was rotated at a constant speed (set 3) at the specified temperature. Aliquots of the mixture (30–40 mL) were removed at one-hour time intervals, centrifuged (5000 rpm for 15 min), decanted, filtered (Whatman paper #2) and analyzed for iodine value (IV), saponification value (SV), peroxide value (PV), and percentage of free fatty acid (FFA, as oleic acid) by following slight modifications of the AOAC procedures (11); carotenoids were determined by a spectrophotometric procedure (12).

The study was conducted as a factorial experiment. However, the factors under evaluation were assigned to experimental units of different size, which created a split-plot experiment design (13). While the treatments from the factorial combination between temperature and concentration of H_2O were distributed among the oil bottles (*i.e.*, whole experimental units), the adsorption time was assigned to the aliquots obtained from the mixture (oil/water/carbon) (*i.e.*, split experimental units) during the adsorption. Therefore, the data were statistically analyzed as a "split-plot" design in which the "whole plot" factors were temperature and concentration of H_2O in the system, and the "split-plot" factor was adsorption time. The STAN program (14) was utilized for linear modeling. The effects of the variables and their interactions were evaluated in single degrees of freedom; when the effect was not significant ($P > 0.10$), its associated sum of squares was pooled into the error term.

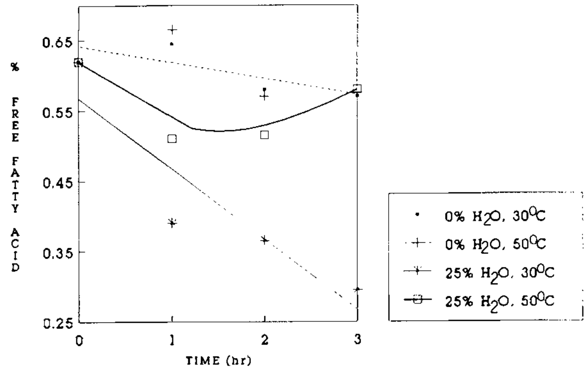

FIG. 1. Effect of adsorption time and water concentration in the adsorption system (0% H_2O and 25% H_2O) at two temperatures (30°C and 50°C) on the concentration of free fatty acid (as % w/w of oleic acid) remaining in *Cucurbita moschata* seed oil after adsorption with activated carbon (5% w/w).

RESULTS AND DISCUSSION

The split-plot experimental design involves two sizes of experimental units; consequently, there are two experimental errors, one for each size of unit (13). Generally, the error associated with the "split-plot" factor is smaller than the "whole-plot" error, mainly because the error degrees of freedom for the "whole error term" are usually less than the degrees of freedom assigned to the "split error term." As a result, the precision involved in the evaluation of the effect of contact time ("split-plot" factor) and its interactions with the effect of temperature and water concentration was improved.

The adsorption process described in this paper was a multicomponent system. In most cases activated carbons have a wide range of affinity for materials in solution, and therefore all adsorbable compounds present in the oil compete for adsorption sites. Additionally, when water is present a three-phase system (*i.e.*, carbon, oil, and water) is established, with subsequent partitioning of the oil components between the two immiscible liquid phases (*e.g.* oil/water). Many polar organic compounds, like fatty acids, phenols, and a variety of pesticides are partially soluble in water (10,15). It can be shown that the solubility and adsorption of a given species are inversely related factors (16). Accordingly, as temperature and the presence of water in the system affect the oil/water partitioning of the oil components as well as their solubility in each phase, these in turn affect the extent of the components' adsorption.

Oil acidity is mainly the result of free fatty acid released from the acylglycerides by enzymatic action and/or the combined action of heat and water (3,17). Figure 1 shows the effect of activated carbon on FFA adsorption in *C. moschata* oil as a function of contact time, temperature, and the concentration of H_2O in the system. The standard deviation ("split error term") for the experiment was 0.045 with 14 degrees of freedom. Although the solubility of the fatty acid decreases as the polarity of the solvent increases, fatty acid oil/water partitioning is anticipated (10,15). Fatty acid solubility in water shows an inverse relationship with the carbon chainlength (10), while fatty acid solubility (*i.e.*, in water or organic solvents) shows a direct relationship with temperature (18). As a result, under the conditions of low fatty acid solubility (*i.e.*, low temperature and fatty acid oil/water partitioning, 30°C and 25% H_2O present in the system), the amount of adsorbed fatty acid was higher than under the conditions of significant fatty acid solubility (*i.e.*, no fatty acid oil/water partitioning and/or high temperature, 0% water in the adsorption system at 30°C and 50°C) (Fig. 1). While at 30°C and 25% H_2O in the adsorption system there was a significant linear adsorption of FFA ($P < 0.01$) as a function of contact time, at 50°C and 25% H_2O the contact time observed a quadratic effect ($P < 0.02$) on the FFA adsorption (Fig. 1); this result suggests that at 50°C and 25% H_2O, some FFA desorption occurred after 2 hr. On the other hand, with 0% H_2O the effect of contact time on the FFA adsorption was not significant at both 30°C ($P > 0.10$) and 50°C ($P > 0.10$) (Fig. 1).

The behavior of the PV was independent of the level of H_2O in the adsorption system ($P > 0.25$); this result seems to indicate that peroxide does not show significant oil/water partitioning. However, the PV was affected by a significant interaction between the effects of contact time and temperature ($P < 0.025$) (Fig. 2). The standard deviation for this experiment ("split error term") was 1.44 mEq/kg of oil with 20 degrees of freedom. At 30°C the PV showed a quadratic behavior ($P < 0.025$) with a maximum at 1 hr of adsorption, while at 50°C the PV decreased linearly ($P < 0.025$) as a function of contact time (Fig. 2). With the exception of the oil at 0 hr of adsorption, the PVs obtained at 50°C were always significantly lower than those obtained at 30°C ($P < 0.05$). It has been sug-

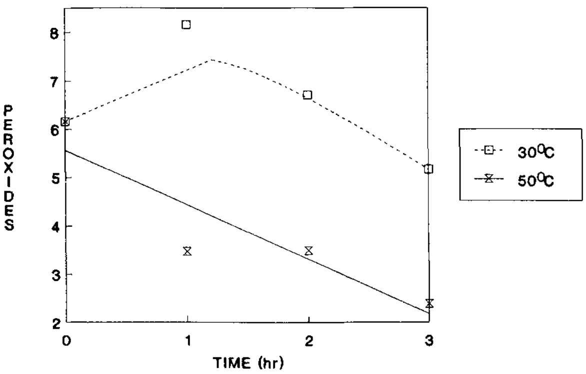

FIG. 2. Effect of adsorption time and temperature on the concentration of peroxide (mEq/kg of oil) remaining in *Cucurbita moschata* seed oil after adsorption with activated carbon (5% w/w).

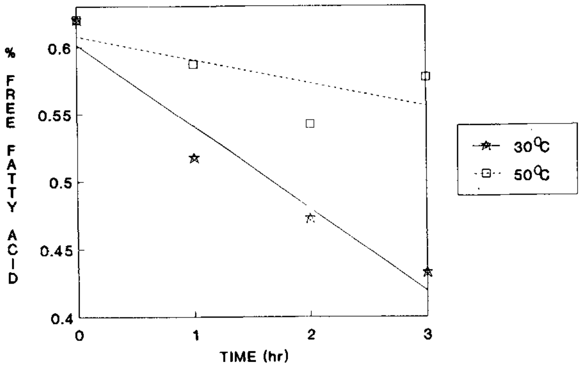

FIG. 3. Effect of adsorption time and temperature on the concentration of free fatty acid (as % w/w of oleic acid) remaining in *Cucurbita moschata* seed oil after adsorption with activated carbon (5% w/w).

gested (8) that the behavior of the PV during the adsorption process is the result of a balance between three processes, namely, peroxide formation (3), peroxide decomposition (3,7,19), and peroxide adsorption (4,9). The results obtained seem to indicate that peroxide adsorption and/or decomposition predominated at 50°C, while peroxide formation predominated at 30°C, especially at low contact times. Using a model system of methyl linoleate and soybean oil, Miyashita and Takagi (20) have shown the prooxidant activity of FFA. The same authors observed that FFA concentration did not decrease as autoxidation proceeded, presumably because the FFA just catalyzed the peroxide homolytic cleavage, accelerating in this way autoxidation by radical chain reaction (20). Figure 3 shows the effect of contact time at 30°C and 50°C on the FFA adsorption, independent of the concentration of H_2O in the adsorption system. While at 30°C there was a significant linear adsorption of FFA ($P < 0.001$) as a function of contact time, at 50°C the adsorption time effect was not significant ($P > 0.25$). Therefore, FFA are significantly adsorbed on the carbon matrix at 30°C (Fig. 3), and this might promote their prooxidant activity. Consequently, they induce peroxide production over peroxide adsorption and decomposition (Fig. 2). Crash *et al.* (21), by using several vegetable oils, also observed shorter induction times for the autoxidation reaction (evaluated by chemiluminescence) when an active matrix interacted with the oils.

The presence of two immiscible phases, oil/water, did not significantly affect the adsorption of carotenoid ($P > 0.10$) in *C. moschata* oil. However, the carotenoid concentration decreases as a quadratic function of the adsorption time ($P < 0.001$) (Fig. 4); this effect was more accentuated at 50°C than at 30°C ($P < 0.025$). At 50°C the carotenoid adsorption equilibrium was attained within 1 hr of adsorption, whereas at 30°C equilibrium was not reached within the time interval studied. The standard deviation for this experiment ("split error term") was 5.69 mg/kg of oil with 20 degrees of freedom. Although a substantial reduction of carotenoid concentration was reached after 1 hr adsorption at both 30°C and 50°C, the remaining oils did not show an acceptable color (evaluated as the lack of appreciable green color when 50 mL of oil were observed in 10 × 100 mm test tubes against a white background).

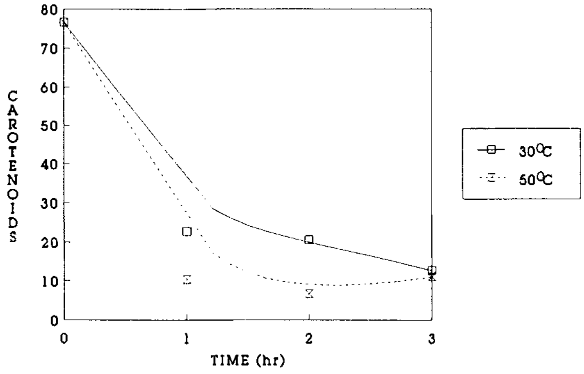

FIG. 4. Effect of adsorption time and temperature on the concentration of carotenoid (mg/kg of oil) remaining in *Cucurbita moschata* seed oil after adsorption with activated carbon (5% w/w).

Neither the temperature or the concentration of H_2O in the adsorption system significantly affected ($P > 0.20$) the behavior of iodine value (IV) during the adsorption process. Figure 5 shows the effect of the adsorption time ($P < 0.005$). The IV decreased after 1 hr; however, further adsorption time did not show additional effect on the IV. The IV is directly associated with the extent of unsaturated fatty acids in an oil or fat; thus, a decrease in IV involves a reduction in the proportion of unsaturated fatty acids in the oil. On the other hand, the saponification value was affected significantly ($P < 0.10$) by contact time and the concentration of H_2O in the adsorption system. The standard deviations ("split error terms") for these experiments were 7.74 (with 21 degrees of freedom) and 27.01 (with 19 degrees of freedom) for IV and SV, respectively. Independent of the temperature, at 0% H_2O concentration SV increased linearly as the adsorption time increased, while at 25% water concentration SV increased, reaching equilibrium after 1 hr of adsorption time (Fig. 5). Phospholipids are acylglycerides that hydrate in the presence of water, rendering them insoluble in oil (1,3); the complete separation of the hydrated phospholipids may be accomplished by centrifugation (1). Additionally,

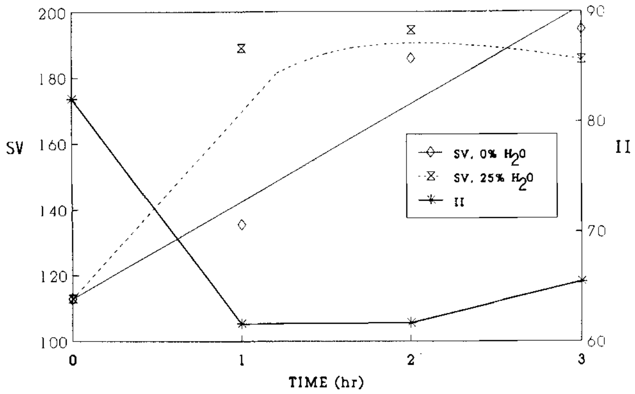

FIG. 5. Behavior of iodine value (IV) and saponification value (SV) of *Cucurbita moschata* seed oil after different adsorption times with activated carbon (5% w/w) in the absence (0% H_2O) or presence of water (25% H_2O).

the SV has an inverse relationship with carbon chain-length of the saponified fatty acid. These points imply that acylglycerides, probably phospholipids, with long-chain fatty acids were adsorbed/precipitated preferentially.

Dutt and Prasad (22) have shown that the IV/SV ratio has a mathematical relationship with viscosity and the function of viscosity with temperature. The results presented in Figure 5 indicate that the IV/SV ratio varied as a function of the adsorption conditions utilized as well as with contact time. This point is in agreement with previous results (8) and suggests that the physicochemical properties, and therefore the functional properties of an oil may be modified as a function of the conditions utilized during the adsorption process.

So far, the only operation in current refining of vegetable oils that makes use of adsorption is the bleaching process. A study of the factors that determine the characteristics of phase partition equilibriums during the adsorption process, and the rates at which these equilibriums are attained during the process of oil adsorption should show the advantages for use of the adsorption phenomenon in the oil refining industry.

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