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# Spectrophotometric analysis of the lipid fraction of microwave heated and soaked soybeans

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**RESUMEN**

**Análisis espectrofotométrico de la fracción lipídica de soja macerada y calentada en microondas.**

Uno de los principales problemas para la aceptación de los productos de la soja por las poblaciones del Occidente es el «flavor», sobre todo de la leche de soja. Con este trabajo se buscó evaluar los efectos del pre-calentamiento con microondas a diferentes tiempos sobre la calidad oxidativa de la soja, midiendo la absortividad a 232 nm de la fracción lipídica después de la maceración para la producción de la leche de soja. Con este método fue posible detectar la disminución de la oxidación durante la maceración y comprobar que fue menor cuanto más largo fue el tiempo de exposición a las microondas. La soja no calentada presentó una absortividad de 5,36 y las calentadas durante 150 segundos, 2,93, a una temperatura final de 142,5°C. Los datos se ajustaron a una ecuación de segundo orden.

*PALABRAS-CLAVE: Calentamiento en microonda — Espectrofotometría UV — Leche de soja — Oxidación — Soja macerada.*

**SUMMARY**

**Spectrophotometric analysis of the lipid fraction of microwave heated and soaked soybeans.**

The flavor of soybean products, particularly soy milk, is one of the main problems to its acceptance by Western people. The objective of this work was to evaluate the effects of different microwave pre-heating times on soybean oxidative quality by monitoring the spectrophotometric absorptivity at 232 nm of its lipid fraction during soaking for soymilk production. A decrease in oxidation level was observed in microwave heated soybeans and the longer the exposure time was, the lower was the absorptivity. Absorptivity of non-heated soybeans was 5,36, while those heated for 150s, reached a temperature of 142,5°C and presented 2.93. Data could be represented by a polynomial equation of second order.

*KEY-WORDS: Microwave heating — Oxidation — Soaked soybean — Soymilk — UV spectrophotometry.*

## 1. INTRODUCTION

During soaking of soybeans for the production of soymilk in the traditional way, great damage occurs to the cells due to the rapid water absorption rate (3) which sets lipoxygenases free to oxidise the lipid fraction yielding that «known bean flavor», so unpleasant to the Western people. Several methods for lipoxygenase inactivation can be found in the literature (3, 5, 12, 17) and do improve soybean products flavor. However, many times, these methods are also responsible for a decrease in extractability of the protein which hinders high yields. A very promising technology is preheating soybean in microwave oven aiming at the inactivation of these enzymes before soaking. Many authors have already worked on the subject and succeeded inactivating lipoxygenases by microwave treating soybeans without reducing NSI (nitrogen solubility index) and producing high nutritional quality soymilk (15, 16, 17). This study was carried out in order to determine by ultraviolet spectrophotometry the total oxidation level (which includes the enzymatic damage, due to lipoxygenases activity), of the lipid fraction of soybean preheated in microwave oven for different periods of times and soaked at room temperature.

## 2. MATERIAL AND METHODS

Davies cultivar soybeans from 94/95 crop were analysed for oil (2), protein (1), ash and moisture contents (9). Peroxide and acid values (2) and $E_{232}$ were determined in the solvent extracted oil (10).

### 2.1. Microwave heat treatment

150g of sound soybeans were placed in three 8.5-9 cm Ø Petri dishes in the centre of the rotative support dish of a Sanyo, EM-804TGR microwave oven. Soybeans were heated at 800W, 2450Mhz, for 30, 60, 90, 120 and 150 seconds. Final temperature was monitored immediately after treatment with a Tecnal Checktemp 1, copper-constantan thermocouple Soybeans were weighed for water loss determination right after cooling at room temperature.

### 2.2. Soaking

Each sample (50g) was immersed in 150g tap water at room temperature (22°C). After eight hours of soaking, grains were drained and freeze-dried.

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### 2.3. Lipid fraction extraction for spectrophotometric examination

Freeze-dried soybeans were milled in cyclone mill and extracted according to Hara & Radin (8) method modified and adapted by Evangelista & Regitano-D'Arce (7). First extraction was performed with 8ml isopropanol (ISP), according to De La Roche & Andrews (6), followed by addition of 12ml isooctane (ISO), instead of hexane-isopropanol mixture (8). 12ml sodium sulphate solution (1g anhydrous $Na_2SO_4$/ 15ml) were added to the miscella collected and stirred in a Potter homogeniser. 5ml of the top layer were collected and transferred to a glass dish for miscella gravimetric oil content determination. 2ml of the same top layer were transferred to a 25ml volumetric bottle filled with 3:2 ISO-ISP mixture for ultraviolet spectrophotometric examination at 232nm (10).

## 3. RESULTS AND DISCUSSION

The composition and oil quality of soybean employed in the experiments (table 1) correlated well with previous works by Mandarino *et al.,* (11) and Turatti *et al.,* (14), with the same Brazilian cultivar.

Table 1
**Davies cultivar soybean composition**

| Components | % p/p |
|---|---|
| Moisture | 9.04 |
| Crude protein[1] | 37.46 |
| Total lipids[1] | 23.87 |
| Ash[1] | 5.41 |
| Carbohydrate[2] | 33.26 |

1 Dry matter basis
2 Calculated from the percentual difference of the other components.

Results from Table 2 assures the good quality of the lipid fraction. Maximum values for crude Brazilian soybean oils are 2% acidity and 20mEq/kg peroxides.

Table 2
**Davies cultivar soybean oil characterisation**

| Analysis | |
|---|---|
| Acid value (% p/p)[1] | 0.76 |
| Peroxide value (mEq/kg) | 1.84 |
| $E_{232\ nm}$ | 3.05 |

1 Expressed in oleic acid.

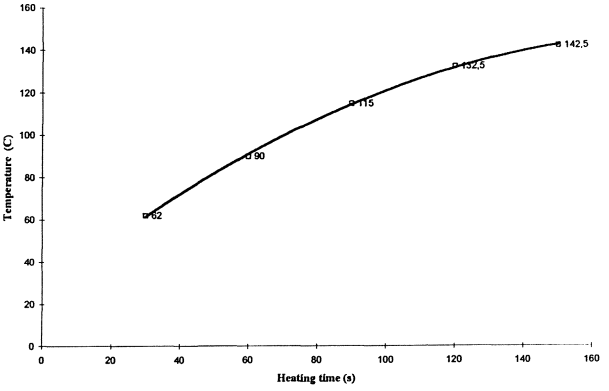


Figure 1
Soybean temperature after heating in microwave oven

Figure 1 presents the final average temperature for various microwave heating times. A similar behaviour was obtained by Pour-El *et al.,* (13). A polynomial equation of second order can be determined for the curve of grain temperature (T) in °C as a function of microwave heating time (t) in seconds:

$$T\ (°C) = -\ 0.0035\ t^2 + 1.2998\ t + 25.6 \quad (R^2 = 0.9995)$$

Temperatures reached in this work were intermediate between those by Pour-El *et al.,* (13) and those obtained by Wang & Toledo (16) for the same exposure time. Our records indicate 132.5°C after 120 seconds in microwave, Pour-El *et al.,* (13) detected 190°C in an American cultivar (7.6% moisture) while Wang & Toledo (16) observed 80°C for Santa Rosa cultivar, with 8.7% moisture. Although microwave frequencies were the same (2450 Mhz) for the three works, differences may exist and may be due to the potency and way of operation of the equipment as well as sample size. This work was conducted at maximum potency (800 W). Moisture losses increased with heating time. Figure 2 shows the percentual loss of grain moisture as a function of microwave heating time. After microwave heating for 150 seconds, soybeans started to toast due to total water evaporation. So the limiting time of heating in Figure 2 was fixed in 150s, when there was still water to be heated and evaporated. Water is the component that mostly absorbs thermal energy during microwave heating.

Pour-El *et al.,* (13) used the energy absorbed (EA) concept as an indicator of the microwave heating effect, considering the energy absorption by the grain during electric heating due to two main processes: grain temperature increase and water evaporation (moisture loss).

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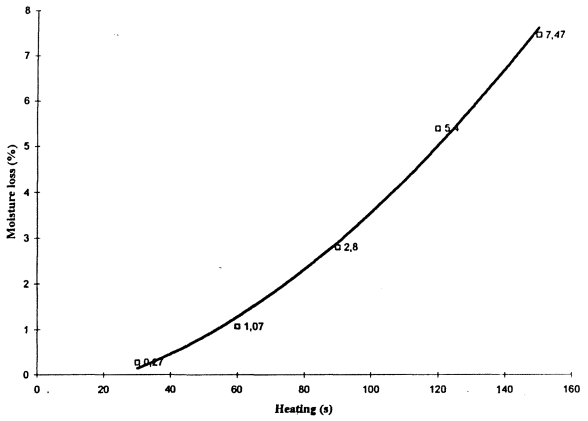


Figure 2
Moisture loss in microwave heated soybeans

Pour-El *et al.*, (13) determined the **MEA** (minimum energy absorbed per gram) values for grains during a dielectric heating, due to temperature raise and water vaporisation. Calculations involved $c_p$ = 0.39123+ + 0.0046057 M, for specific heat and $h_v$ = 539 (1 + + 0.21624 $e^{-0.06233\,M}$) for grain water vaporisation heat, where M is grain moisture in dry basis: MEA = $c_p$ $\Delta T$ + + $h_v$ $\Delta M$, where $\Delta T$ is the temperature rise and $\Delta M$ is the percentual moisture loss (dry basis). From that study, it was observed that 90% of lipoxygenase activity was lost at a MEA of 150 cal/g. For 100 cal/g MEA, less than 40% of the original enzyme activity remained. Data from Figures 1 and 2 were included in the following MEA calculations for the present study (Table 3).

Table 3
**Microwave heated soybeans Minimum Energy Absorbed (MEA)**

| Time (s) | $\Delta M$ (%)[1] | $\Delta T$ (°C)[2] | MEA (cal/g)[3] |
|---|---|---|---|
| 30 | 0.30 | 42 | 20 |
| 60 | 1.17 | 70 | 38 |
| 90 | 3.08 | 95 | 60 |
| 120 | 5.94 | 112 | 85 |
| 150 | 8.21 | 122 | 103 |

1 Dry matter basis

2 Initial temperature ~20 °C

3 $C_p$ = 0,44 cal/g °C e hv = 602 cal/g

Present experimental data did not reach 150 MEA, but surpassed 100. Periods of heating of 120 and 150s were very efficient in lowering the level of oxidation. The longer the heating period in the microwave oven, the higher the amount of water evaporated and the temperature reached, therefore, the higher was the energy absorbed.

Wang & Toledo (17) detected 2.3% residual lipoxygenase activity after 240 seconds of heating and 122.3°C final temperature. Data from Figure 1 indicates that similar temperature was reached after 90 seconds and therefore lower residual enzyme activity, and therefore lower oxidation level, should be expected. Data from Figure 3 confirms this observation because absorptivities after 90 seconds of heating reached values close to those of the intact oil (lipid fraction) from non-heated soybeans (3.05), from Table 2. UV absorptivities indicate lipid oxidation level.

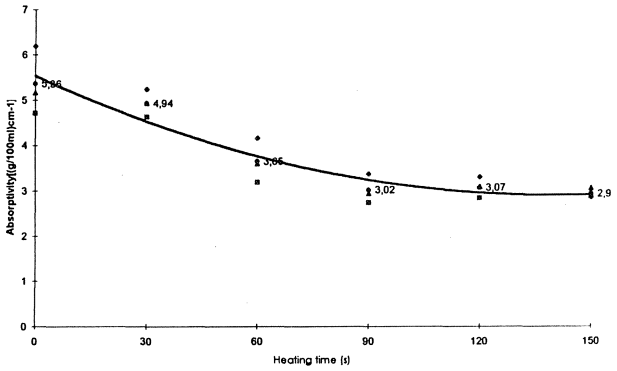


Figure 3
Absorptivity of microwave heated and soaked soybean lipid fraction at 232nm

Figure 3 indicated that heating periods longer than 90s prevented oxidation in soybeans. Spectrophotometric data adjusted to a polynomial equation of second order:

$$\text{Absorptivity} = 0.0001\ t^2 - 0.0376\ t + 5.5443 \quad (R^2 = 0.9515)$$

## 4. CONCLUSIONS

The use of the modified Hara & Radin method for microwave heated soybean lipid fraction extraction was simple, quick and of low cost, allowing direct analysis of the miscellae by spectrophotometry for the determination of the oxidation degree and, indirectly, the enzymatic activity level in soybeans. This study confirmed that microwave heating of soybeans reduces oxidation during soaking and that

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the longer the heating period, the lower is the oxidation in soybean lipid fraction.

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