Artículo Original / Original Article

Thermal stability of avocado oil: A comparative study with rice bran and olive oils

Estabilidad térmica del aceite de avocado: Un estudio comparativo con los aceites de arroz y oliva

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Este trabajo fue recibido el 14 de enero de 2021

Aceptado con modificaciones: 30 de marzo de 2021.

Aceptado para ser publicado: 10 de mayo de 2021.

ABSTRACT

This study aimed to evaluate the thermal stability of avocado oil (Breda variety) compared to olive and rice bran oils. The oils were heated to a temperature of 180 °C for 1.5, 3.0, 4.5, and 6.0 h in a digester block. The evaluations performed were: acidity, indices of peroxides, iodine, p-anisidine, refraction, electrical conductivity, specific extinction coefficients, phenolic compounds, chlorophylls, carotenoids, and antioxidant activity of the samples. Changes in the evaluated parameters were found as a function of the heating time. In general, olive oil was found to be more stable, followed by avocado oil. However, the avocado oil showed good maintenance of characteristics when subjected to heating. This work has shown that avocado oil is very similar to olive oil and that both show considerable stability after heating at the usual temperature of the frying process.

Keywords: Bioactive compounds; Heating; Lipids; Quality; Stability.

RESUMEN

El objetivo de este estudio fue evaluar la estabilidad térmica del aceite de aguacate (variedad Breda) en comparación con los aceites de salvado de arroz y oliva. Se calentaron los aceites a una temperatura de 180 °C durante 1,5; 3,0; 4,5 y 6,0 h en un bloque digestor. Las muestras fueron sometidas a los siguientes análisis: acidez, índices de peróxidos, yodo, p-anisidina, refracción, conductividad eléctrica, coeficientes de extinción específica, compuestos fenólicos, clorofilas, carotenoides y

actividad antioxidante. Se encontraron cambios en los parámetros evaluados en función del tiempo de calentamiento. Por lo general, se encontró que el aceite de oliva es más estable, seguido del aceite de aguacate. Sin embargo, el aceite de aguacate mostró un buen mantenimiento de las características bajo el calentamiento. Este trabajo ha demostrado que el aceite de aguacate es muy similar al aceite de oliva y que ambos muestran una estabilidad considerable al calentar a la temperatura habitual del proceso de fritura.

Palabras clave: Calentamiento; Calidad; Compuestos bioactivos; Estabilidad; Lípidos.

INTRODUCTION

Avocado stands out compared to other fruits because it is of the most complete one in terms of nutritional quality. The protein content in its pulp ranges from 1 to 2%, sugars range from 3 to 8% and oil from 5 to 35%, being characterized as one of the only fruits rich in lipids^{1,2,3}. This fruit has a high content of oleic acid (omega-9) and unsaturated fatty acids, which are associated with a reduction in the risk of coronary heart disease^{3,4}. It also contains several minerals and vitamins, especially potassium, vitamin C and E (α -tocopherol and γ -tocopherol), besides lutein and b-carotene^{1,3}.

Avocado is widely consumed *in natura*. On the other hand, avocado oil is among the main industrial products of the fruit. Avocado oil contains several health-beneficial lipophilic compounds, such as phytosterols and other bioactive compounds, including carotenoids, aliphatic alcohols, terpene alcohols, tocopherols, and squalene⁵. Both the avocado fruit and its oil are associated with protective effects, reducing the risk of coronary heart disease, cataract, diabetes and prostate cancer. Some of these effects are attributed to the antioxidant pigments present in the fruit³.

The oil extracted from avocado has several uses, both in the manufacture of cosmetics and in pharmaceuticals. Also, it is used in the food industry, in the enhancement of products and for direct consumption⁶. However, the direct consumption of avocado oil in human food is still not common, despite the many health benefits associated with this product⁷.

Avocado oil is very similar to olive oil, both in its manner of production and in the physicochemical characteristics. These oils are extracted from the fruit pericarp by physical processes (mainly centrifugation), do not undergo refining processes and are also considered sources of compounds with beneficial effects on human health, such as unsaturated fatty acids, vitamin-E, phenolic compounds, sterol and carotenoids. Oleic acid is the major element in both oils^{8,9}.

Heating oils at high temperatures and for long periods may change their composition by degrading fatty acids and other minority compounds. Knowledge of the thermal properties of oils is fundamental to define their application in any technological process accurately. This, in turn, allows the adoption of better strategies for the conservation of structure, as well as their sensory and technological characteristics¹⁰.

Data report that olive oil shows good stability, even at high temperatures (such as those required for frying), and also preserves its beneficial characteristics¹¹.

One study reports a similarity between the thermal behavior of olive and avocado oils¹². However, there is very

little information regarding the latter, especially about the effect of temperature on the quality and pigments parameters. These facts motivate research in the field of the effect of heating on avocado oil characteristics. We evaluated rice bran and olive oils for comparison. Rice bran is a reference for good stability and it is recognized for its high smoke point. Olive oil is a similar product. Thus, the aim of this work was to evaluate the stability of avocado oil when subjected to heating at 180 °C, at different exposure times, compared to olive oil and rice bran oil.

MATERIALS AND METHODS

The samples of Breda avocado (*Persea americana* Mill) oils were donated by a producer from São Sebastião do Paraíso/MG/Brazil. Extra virgin olive oil, produced in Buenos Aires, Argentina, and refined rice bran oils, produced in Pelotas/RS, Brazil, were obtained from the local market.

Oil samples were heated in a digester block (Marconi, model MA 850, Brazil) at a temperature of 180 °C for the time points of 1.5, 3.0, 4.5, and 6.0 h. An unheated sample was used (time 0) as a control.

The samples were stored in 10 mL amber flasks and maintained in a ULT freezer (Coolclab CL120–86V, Brazil) until analysis.

Stability after heating was evaluated by the determination of the following parameters: indices of acidity, peroxide, iodine, p-anisidine and refractive; electrical conductivity; specific extinction coefficient; total phenolic compounds; carotenoid and chlorophyll content, and antioxidant activity.

A randomized experimental design in a 3×5×12 factorial scheme was used, consisting of three samples (avocado oil, rice bran oil, and olive oil), five evaluation periods (time of exposure to heating) and twelve types of evaluations (dependent variables).

The acidity (Ca 5a-40), peroxide (Cd 8b-90), iodine (Cd1-25), refractive (Cc 7-25) and p-anisidine (Cd 18-90) indices were determined according to AOCS indications¹³. The electrical conductivity was determined using a conductivity meter (Tecnopon, Brazil) at 25 °C and expressed in $\mu\text{S}\cdot\text{cm}^{-1}$.

The specific extinction coefficient was determined by IOOC method¹⁴, at 232 nm and 270 nm (spectrophotometer Jenway 6705 UV/VIS, UK).

Phenolic compounds were extracted using the method described by Montedoro et al.¹⁵, using a solution of methanol/water (80:20 v/v), agitation and two-stage centrifugation (15.000 and 5.000 g). The determination was performed by the colorimetric reaction described by Gambocorta et al.¹⁶, using the Folin-Ciocalteu reagent, at 750 nm. For

quantification, a standard gallic acid curve was constructed, containing 5 points ($y = 0.0082x + 0.0179$ / $R^2 = 0.9919$).

Total chlorophylls and carotenoids were determined by the methodology of Rodrigues-Amaya¹⁷, using an isooctane:ethanol (3:1) solution. The absorbance was recorded at 450 nm for carotenoids and at 630, 670 and 710 nm for chlorophylls.

The antioxidant activity was determined using the ABTS radical inhibition method (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), according to the methodology described by Re et al.¹⁸, at 734 nm.

All determinations were performed with at least 3 replicates. The comparison between the samples was performed by analysis of variance, complemented by Tukey's test, at 5% significance level, using the Statistix 10 program. Changes in samples after heating at different times were evaluated by polynomial regression, because in general, this model was the one that best fitted the data.

RESULTS

Acidity Content

The acidity determination showed that unheated avocado oil sample had significantly higher acidity ($p \leq 0.05$) than the other oils without heating (Table 1). After 1.5 h of heating, it was found that the acidity of all samples increased expressively; the acidity of avocado oil was similar to that of rice bran oil ($p \leq 0.05$), and significantly higher than of olive oil ($p \leq 0.05$) (Figure 1A and Table 1). This behavior was maintained at the highest heating times, although values became numerically closer to each other at the end of the heating time.

During heating (Figure 1A), maintenance of acidity was observed in avocado oil. Numerical reduction was observed in some values between 1.5 and 6.0 h, which was not significant. Changes observed in acidity with oil heating were not considered significant ($R^2 \leq 0.9$), after statistical evaluation by polynomial regression, because the initial acidity of avocado oil was high. However, it did not increase in the same proportion as the other samples.

In rice bran and olive oils, there was a trend of progressive increases in this parameter: the statistical evaluation of stability was found to be significant for both.

Iodine index

Comparing the samples at each heating time, significantly higher values ($p \leq 0.05$) of I.I. were observed for all time points for rice bran oil (Table 1). Avocado and olive oil were not significantly different.

The evaluation of iodine index for each sample over the heating time showed that the changes were not significant in any lipid samples ($R^2 \leq 0.9$) (Figure 1B).

Peroxide index

Among the control samples (not heated), olive oil showed the highest value ($p \leq 0.05$) of peroxide index. After 3 h of heating, rice bran oil showed significantly higher peroxide index ($p \leq 0.05$) than that of olive oil and avocado oil, maintaining this behavior until the end of the heating period (Table 1). However, despite the significant difference between the peroxide indices during

heating, all samples presented low values for the primary oxidation products (peroxides and hydroperoxides).

Olive oil showed a gradual reduction in its peroxide content, while the other two oils increased during heating (Figure 2A and Table 1).

A significant reduction in the peroxide index was verified in olive oil while evaluating the primary oxidation products over the heating times; changes in the peroxide index of the other two samples were not significant.

p-Anisidine index

Significant differences were observed in the p-anisidine index among the control (unheated) samples, with rice bran oil showing the highest value and avocado oil showing the lowest ($p \leq 0.05$). Beginning at 1.5 h until the end of the heating time, avocado oil and olive oil showed no difference in their p-anisidine index, and their values were significantly lower than those obtained for rice bran oil ($p \leq 0.05$) (Table 1).

When the products were evaluated over the heating period, none of them showed a significant change in this parameter (Figure 2B).

Refractive index

No variation was observed in the refractive index between replicates of the same sample; therefore, nullifying the need for the application of the means test. Among the control samples, rice bran oil showed the highest refractive index (Table 1). This could be due to the fact that rice bran oil contains higher levels of unsaturated fatty acids compared to avocado and olive oils. At 1.5, 3.0, 4.5 and 6.0 h, the RI did not change much.

The RI of avocado oil increased after 1.5 h of heating, remained stable for 4.5 h and then decreased after 6 h. A reduction in RI of rice bran oil was observed with heating; this was maintained after 3 h until the end of the heating process. No clear trend was observed for olive oil. However, these changes were not significant for any of the samples over the course of heating (Figure 3A).

Electric conductivity

In relation to electrical conductivity no differences were observed in values as a function of heating for any of the oil samples (Figure 3B and Table 1).

Specific extinction coefficient (K_{232} and K_{270})

The rice bran oil showed significantly higher values than the others ($p \leq 0.05$) at all heating time points (Table 1). Olive oil showed lower values ($p \leq 0.05$). There was a difference in the K_{270} values between rice bran oil and the other two oils, with rice bran oil showing significantly higher values than the others ($p \leq 0.05$) (Table 1). Statistically significant differences were observed in the values of K_{232} between the samples; however, numerically, the values were similar.

The evaluation of K_{232} and K_{270} in oils over the heating time (Figure 4A and 4B) indicated a significant change in the rice bran oil, but only at the wavelength of 270 nm (Figure 4B). A small reduction in values was observed with heating.

Table 1. Effect of heating on the quality parameters of avocado, rice bran, and olive oils.

Samples	Time (hours)				
	0	1.5	3.0	4.5	6.0
Acidity (% oleic acid)					
Avocado Oil	1.08±0.14 a	3.64±0.31 a	3.23±0.31 a	3.02±0.11 a	3.44±0.10 a
Rice Bran Oil	0.09±0.01 b	2.61±0.10 a	2.81±0.11 ab	3.02±0.09 a	3.22±0.10 ab
Olive Oil	0.19±0.01 b	2.28±0.20 b	2.40±0.31 b	2.60±0.11 b	2.92±0.21 b
Iodine index (g de I ₂ .100 g ⁻¹)					
Avocado Oil	89.07±0.15 b	85.89±4.022 b	87.92±0.78 b	84.16±3.45 b	87.90±1.98 ab
Rice Bran Oil	111.21±4.83 a	97.43±1.09 a	97.16±3.93 a	100.32±0.43 a	90.69±10.36 a
Olive Oil	93.29±3.05 b	80.81±0.97 b	86.99±3.80 b	81.37±1.55 b	74.93±0.19 b
Peroxide index (mEq-g O ₂ .kg ⁻¹)					
Avocado Oil	6.28±0.49 b	6.91±1.77 ab	6.12±0.20 b	7.51±0.59 b	8.09±0.99 b
Rice Bran Oil	4.27±0.30 c	4.84±0.10 a	9.77±0.11 a	11.25±0.39 a	10.07±0.19 a
Olive Oil	8.50±0.30 a	8.44±0.30 b	8.24±0.29 c	7.40±0.31 b	7.34±0.16 b
p-Anisidine index					
Avocado Oil	0.22±0.02 c	0.24±0.00 b	0.23±0.00 b	0.24±0.00 b	0.15±0.03 b
Rice Bran Oil	0.55±0.00 a	0.60±0.16 a	0.75±0.03 a	0.70±0.07 a	0.79±0.00 a
Olive Oil	0.27±0.00 b	0.24±0.00 b	0.23±0.03 b	0.24±0.00 b	0.15±0.00 b
Refractive index					
Avocado Oil	1.462±0.000	1.464±0.000	1.464±0.000	1.464±0.000	1.462±0.000
Rice Bran Oil	1.465±0.000	1.464±0.000	1.462±0.000	1.462±0.000	1.462±0.000
Olive Oil	1.464±0.000	1.464±0.000	1.462±0.000	1.464±0.000	1.462±0.000
Conductivity (μS.cm ⁻¹)					
Avocado Oil	0.18±0.00	0.18±0.00	0.18±0.00	0.17±0.00	0.17±0.00
Rice Bran Oil	0.17±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.17±0.00
Olive Oil	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00
Specific extinction K ₂₃₂					
Avocado Oil	2.19±0.03 b	2.33±0.04 a	2.29±0.01 b	2.28±0.00 b	2.27±0.00 b
Rice Bran Oil	2.56±0.00 a	2.36±0.07 a	2.40±0.00 a	2.43±0.00 a	2.40±0.00 a
Olive Oil	2.21±0.03 b	2.20±0.00 b	2.21±0.01 c	2.18±0.00 c	2.18±0.00 c
Specific extinction K ₂₇₀					
Avocado Oil	0.17±0.01 b	0.18±0.00 b	0.18±0.00 b	0.17±0.00 b	0.17±0.00 b
Rice Bran Oil	1.98±0.04 a	1.99±0.00 a	1.98±0.00 a	1.96±0.00 a	1.95±0.00 a
Olive Oil	0.16±0.01 b	0.17±0.00 b	0.17±0.00 b	0.17±0.00 b	0.17±0.00 b

Measurements followed by the same lowercase letters in the column do not differ from each other by Tukey's test (p≤0.05).

Figure 1: Acidity index (% oleic acid) (A) and iodine index (I_2 100 g $^{-1}$) (B), of the avocado, rice bran, and olive oils over the heating time at 180 °C.

Figure 2: Peroxide index (mEq-g O_2 ·kg $^{-1}$) (A) and p-anisidine index (B) of the avocado, rice bran, and olive oils over the heating time at 180 °C.

Figure 3: Refractive index (A) and conductivity (B), of the avocado, rice bran, and olive oils over the heating time at 180 °C.

Figure 4: Specific extinction coefficient, K_{232} (A) and K_{270} (B), of the avocado, rice bran, and olive oils over the heating time at 180 °C.

Pigments: Carotenoids and Chlorophylls

Significant differences were observed for chlorophyll and carotenoid contents among the oil samples at all evaluation times. Avocado oil showed significantly higher concentrations ($p \leq 0.05$) of both pigments in all the analysis periods (Table 2).

Lower chlorophyll content was obtained in rice bran oil when compared to olive oil. However, the opposite trend between these oils was seen with respect to carotenoids, where higher content was observed in the rice bran oil, which was maintained even after heating the samples for 6 h at 180 °C (Table 2).

Table 2. Effect of heating on bioactive compounds and antioxidant activity of avocado, rice bran, and olive oils.

Samples	Time (hours)				
	0	1.5	3.0	4.5	6.0
Chlorophylls (mg.kg ⁻¹)					
Avocado Oil	10.44±0.11a	6.11±0.47a	4.27±0.00a	4.43±0.09a	2.12±0.51a
Rice Bran Oil	0.04±0.05c	ND	ND	ND	ND
Olive Oil	1.06±0.00b	0.28±0.14b	0.50±0.01b	0.42±0.00b	0.30±0.05b
Carotenoids (mg.kg ⁻¹ de β-carotene)					
Avocado Oil	20.25±0.08a	16.06±0.00a	13.31±0.01a	11.50±1.42a	10.18±0.15a
Rice Bran Oil	1.52±0.02c	1.86±0.28b	1.19±0.01b	1.13±0.00b	1.61±0.01b
Olive Oil	1.76±0.04b	0.75±0.17c	0.88±0.02c	0.95±0.08c	0.75±0.00c
Antioxidant capacity (% of inhibition)					
Avocado Oil	4.10±0.00b	2.50±0.00a	2.73±0.00a	1.40±0.00a	1.30±0.00b
Rice Bran Oil	4.20±0.00a	1.50±0.00ab	1.30±0.00b	2.00±0.00a	1.70±0.00a
Olive Oil	4.13±0.00ab	2.00±0.00b	1.10±0.00b	1.00±0.00a	1.10±0.00b
Phenolic compounds (mg.kg ⁻¹ of gallic acid)					
Avocado Oil	148.79±0.36b	157.73±3.51b	152.96±3.09b	152.78±1.36b	126.90±1.28b
Rice Bran Oil	120.13±0.12c	118.09±0.97c	121.98±0.24c	122.69±0.08c	125.25±0.88b
Olive Oil	166.41±0.18a	213.47±0.30a	174.78±0.87a	191.92±2.2a	203.07±1.51a

Measurements followed by the same lowercase letter in the column do not differ from each other by Tukey's test ($p \leq 0.05$).

ND: not detectable.

Over the period of heating, avocado oil showed a significant loss of chlorophylls. On the other hand, statistical evaluation by regression (considering $R^2 \geq 0.9$) did not indicate any significant change in chlorophyll content of olive oil and rice bran oil during heating (Figure 5B).

After heating, the carotenoids were significantly lost from olive and avocado oils. No loss in the carotenoids was detected in the rice bran oil; rather, the minor increase observed in the carotenoid levels could be associated with analytical errors, considering the small values of the determinations. The loss in the carotenoid content of olive and rice bran oils during heating was not significant (Figure 5A).

Antioxidant Capacity – ABTS

Some significant differences were observed in the antioxidant capacity among the samples, a slightly higher value was obtained for rice bran oil in the control sample. After 1.5 h of heating, some loss in the antioxidant capacity was observed in all samples: a fact that intensified moderately after 3 h of heating. As heating continued, no further change

in the antioxidant capacity of the samples could be seen, and no clear trend was seen (Table 2).

Statistical evaluation by regression showed a significant change in the antioxidant capacity of olive oil during heating (Figure 6A).

After 6 h of heating at 180 °C, the changes observed in the antioxidant capacity were not significant for avocado and rice bran oils.

Phenolic compounds

A comparative evaluation of the oil samples for their phenolic contents showed significantly higher values in the olive oil ($p \leq 0.05$), both in the control sample and those heated for different times. The avocado oil followed olive oil in phenolic content, while rice bran oil, in general, showed the lowest values for these compounds (Table 2).

Changes in the phenolic content of the heated samples were significant only for avocado oil. Rice bran oil and olive oil showed no significant changes in phenolic content upon heating (Figure 6B).

Figure 5: Carotenoid (mg β-carotene.kg⁻¹) (A) and chlorophyll (mg.kg⁻¹) (B) content of the avocado, rice bran, and olive oils over the heating time at 180 °C.

Figure 6: Antioxidant capacity (% inhibition) (A) and phenolic compounds (mg.kg⁻¹) (B) of the avocado, rice bran and olive oils over the heating time at 180 °C.

DISCUSSION

Acidity content

Acidity content is directly correlated with the quality of oils and fats. It indicates the content of free fatty acids; therefore, it is related to the intensity of hydrolysis of triacylglycerol chains¹⁹.

Overall, olive oil showed significantly lower acidity values than others: probably, the minority compounds that make up the unsaponifiable fraction of olive oil contributed to its greater hydrolytic stability.

The Codex Alimentarius⁹ allows a maximum of 0.8% of oleic acid to the acidity of extra virgin olive oil, and 0.3% of oleic acid to the acidity of rice bran oil. For avocado oil, no such values are defined by legislation. However, due to the similarities in the characteristics and method of production, comparisons were made with the reference values of olive oil. Considering these values, only unheated samples (controls) were in accordance with the legislation. After 1.5 h of heating, all samples showed higher acidities than the maximum values established.

After 6 h of heating, avocado oil showed a 3.2-fold increase with respect to initial acidity, rice bran oil showed a 36-fold increase, while olive oil showed a 15-fold increase. Thus, although all samples showed an increase in acidity, the proportion of increase was the smallest for avocado oil.

An increase in the acidity of the sample demonstrates the occurrence of deterioration processes such as hydrolysis of the triacylglycerols, which gets accelerated by both light and heat²⁰.

Vergara et al.²¹ compared the behavior of soybean oil and rice bran oil after successive potato frying events. They observed that after the eighth frying event, corresponding to a time of 40 min and a temperature of 180 °C, acidity increased by 0.33% in soybean oil, and by 0.14% in rice oil. Values higher than these have been obtained in the current study.

Iodine index

Iodine index is an indication of the extent of halogenation reactions. It is assumed that each double bond present in unsaturated fatty acids has the ability to react with iodine (in the form of monochloride or monobromide) and produce saturated derivatives. Thus, the higher the iodine index, the greater the number of double bonds present in the oil. The importance of determining iodine index lies in its estimation of the content of unsaturated fatty acids, and its correlation with susceptibility to oxidative rancidity, control over the hydrogenation process and/or verification of oil adulteration²¹.

The higher values ($p \leq 0.05$) of iodine index were observed for rice bran oil. This was expected, as this oil has higher levels of linoleic acid (desaturated) than avocado and olive oils^{2,4,9,22}.

The similarity in composition was reflected in the iodine index data, where no significant differences ($p \geq 0.05$) were observed between avocado and olive oil, either in

the controls or in any of the heated samples in the digester block (Table 1).

The established values for iodine index are between 75 and 94 $I_2 \cdot 100 \text{ g}^{-1}$ for extra virgin olive oil⁹, while for rice bran oil are between 90 and 105 $I_2 \cdot 100 \text{ g}^{-1}$ ²³. The results obtained in the current study are, in general, within the recommended limits.

Avocado oil showed the least numerical variation in iodine index over the range of heating times (initial 89.07 and final 87.90 $\text{g } I_2 \cdot 100 \text{ g}^{-1}$); while rice bran oil showed the greatest change (initial 111.21 and final 90.69 $\text{g } I_2 \cdot 100 \text{ g}^{-1}$). The proportion of change in iodine index for olive oil was similar to those obtained for rice bran oil (initial 93.29 and final 74.93 $\text{g } I_2 \cdot 100 \text{ g}^{-1}$). However, these changes were not statistically significant.

The reduction in the iodine index indicates a decrease in the number of double bonds, which, eventually, may be related to changes in constituent fatty acids²¹.

Peroxide index

The peroxide index directly correlates with the oxidation state of oils and fats, as peroxides are the first compounds formed in lipid deterioration²¹.

It is possible that heating resulted in the loss of some of the oxidation products, since olive oil showed a reduction in its peroxide index. Nevertheless, this phenomenon should have affected all the oil samples equally. However, this was not observed in the current study. There was an unequal reduction in peroxide content, presumably because of the protective effects of antioxidant compounds naturally present in olive oil that reduced the rate of peroxide formation.

According to indications in the Codex Alimentarius⁹, the peroxide content of extra virgin olive oil cannot exceed 20.0 $\text{meq. O}_2 \cdot \text{kg}^{-1}$. Thus, this limit was maintained in the current study. No such data are defined in legislation for avocado oil. However, based on the values established for olive oil, it could be considered that the peroxide content of avocado oil remained within recommended limits even after 6 h of heating at 180 °C. For refined oils as rice bran oil, the maximum value of peroxide content can be 10.0 $\text{meq. O}_2 \cdot \text{kg}^{-1}$ ²³. Rice bran oil showed an oxidation state close to its limit after 3 h of heating at 180 °C.

Also, it should be considered that in the fatty acid composition of olive oil (similar to avocado oil), the percentage of unsaturated acids is smaller than that of rice oil^{4,8,22}, a fact that may contribute to greater stability.

Salgado et al.²⁴ evaluated the peroxide content of avocado oil (*Persea americana* mill) extracted with hexane and acetone (1:1) and obtained higher peroxide values than those found in this study (20.6 $\text{meq. O}_2 \cdot \text{kg}^{-1}$).

Cheikhousman et al.²⁵ analyzed two types of extra virgin olive oils, and noted that pre-formed hydroperoxides decreased exponentially during the heating process as a result of their degradation into peroxidation by-products.

p-Anisidine index

The p-anisidine index depicts the content of lipid oxidation by-products, especially aldehyde compounds like p-anisidine, and the subsequent determination of their absorbance at 350 nm. The p-anisidine value is taken as the absorbance multiplied by 100, detected in gram solution of oil in solvent and reagent mixture in a cuvette of 1 cm¹⁴.

No range has been established for the p-anisidine content by the Codex Alimentarius legislation. However, values below 10 indicate that the lipid sample does not contain a significant amount of the secondary oxidation compounds²⁶. All samples in the current study showed reduced content of oxidation by-products, after 6 h of heating in the digester block.

Avocado and olive oils had very similar behavior in relation to the p-anisidine index, showing significantly lower values than rice oil at all time points evaluated.

Refractive index

The refractive index relates to the degree of fatty acid bonding, which increases with the increase in the number of double bonds and also with the increase in molecular mass of fatty acids. This index is also affected by other factors, such as free fatty acid content, the presence of oxidation products, and analysis temperature²¹.

Jorge et al.²⁷ mentioned that the heating may influence the values of the refractive index by breaking the double bond character and hence, leading to a reduction in the values. However, polymerization of some compounds could increase this index.

The recommended refractive index values are between 1.4677 and 1.4705 for extra virgin olive oil⁹ and between 1.460 and 1.473 for rice bran oil²³. The refractive index values obtained for rice bran oil in the current study are in accordance with the above recommendation. However, the refractive indices of other oil samples, regardless of their heating (unheated controls) times, were slightly below recommended ranges.

Varela et al.²⁸ mentioned that the refractive index values of some vegetable oils (soybean, olive, and sunflower) used in repeated frying did not change during the heating process. According to them, in general, this index does not vary when the experimental conditions are milder.

Damy et al.²⁹ determined the physical and chemical changes in soybean oil and hydrogenated vegetable fat occurring during the discontinuous potato frying process. Oil samples were collected after 0.5, 3.5, 5.5, and 7.5 h of frying, and the following analytical determinations were performed: acidity index, total polar compounds (CPT), peroxide index, and refractive index. An increase in the values of these parameters was observed, thus demonstrating that they were good indicators to evaluate the level of change in oils.

Electric conductivity

The electrical conductivity of vegetable oils may be correlated with the presence of decomposition products like

free fatty acids, peroxides, hydroperoxides, aldehydes, and low molecular weight ketones because they can increase the electrical conductivity of the medium²⁶. However, no differences were observed, probably because of the low permittivity (high resistance) of vegetable oils. Moreover, from the data obtained for the peroxide and p-anisidine indices, it could be inferred that there was no expressive production of these species (primary and secondary oxidation products) that could increase the electrical conductivity.

Specific extinction coefficient (K_{232} and K_{270})

The specific extinction coefficient assessed at 232 nm (K_{232}) correlates with the primary oxidation products generated by the conjugation of polyunsaturated fatty acids. The specific extinction coefficient evaluated at 270 nm (K_{270}) is associated with the content of secondary oxidation products.

Rice bran oil presented high values for K_{270} , indicating the presence of secondary oxidation products. But, upon heating, some of these compounds would have been lost due to their low molecular weight and/or volatile nature, since a small reduction in values was noticed with heating.

The Codex Alimentarius⁹ indicates standard values of specific extinction coefficients (K_{232} and K_{270}) only for olive oil; the K_{232} value for the category extra virgin oil must be less than or equal to 2.50, and K_{270} value should be less than or equal to 0.22. All samples (under all heating conditions) in the current study remained within the recommended acceptable range of primary oxidation products (K_{232}). On the other hand, higher values (than those recommended) for the secondary oxidation products (K_{270}) were obtained in the rice bran oil samples, indicating the presence of higher than ideal levels of carbonyl derivatives (aldehydes and ketones) considering the parameter established for olive oil.

According to the literature, moderate thermal processing causes mild changes in the values of extinction coefficients (K_{232} and K_{270}), whereas usual heating temperatures during frying result in the increase of both coefficients^{30,31}.

Comparing the data obtained in the evaluation of the quality and identity parameters of the samples, it can be seen that rice oil showed the greatest losses with heating. Regarding the initial condition, this was the sample with the most marked increase in acidity, that is, the one that most suffered hydrolysis of the triacylglycerols. The presence of free fatty acids must have contributed to the occurrence of oxidative reactions, since the two samples with the highest acidity (rice and avocado oils) also had the highest peroxide index values.

Corroborating the effect of the oxidative process (peroxide index), in which the double bonds of unsaturated fatty acids are compromised (by the reaction with oxygen), the iodine index data also showed that rice oil suffered the most marked reduction in the content of double bonds.

Although the specific extinction coefficient K_{232} is also associated with primary oxidation processes, which lead to the formation of peroxides and hydroperoxides, the values

of this determination did not follow the tendency shown by the determination of acidity, peroxide index and iodine index. However, rice oil has always shown significantly higher values than other oils for K_{232} .

Regarding the parameters related to secondary oxidation products (p-anisidine index and K_{270}), the worst condition of rice oil was again evidenced, which was the only sample with an increase in the p-anisidine index values (about 30% after 6 h of heating). Also, this oil always presented values significantly higher than the others in relation to K_{270} , reinforcing the worst condition in relation to oxidative stability.

Although the increase in free fatty acid content (% acidity) of avocado oil was less intense, in 6 h of heating these hydrolysis products were present in similar quantities in rice and avocado oils. However, this fact did not influence the formation of oxidation products in the same way, either primary or secondary (expressed by the PI, p-anisidine index, K_{232} and K_{270}). This was possibly due to the greater presence of bioactive compounds in the avocado oil, which may have benefited the stability of this oil. The compounds of the unsaponifiable fraction may also have protected olive oil, since it showed a behavior similar to that of avocado oil.

Pigments: Carotenoids and Chlorophylls

Over the period of heating, avocado oil showed a significant loss of about 80% of chlorophylls after 6.0 h of heating at 180 °C. This loss was around 72% in the olive oil sample. The very small concentration of chlorophyll present in the rice bran oil control (unheated) sample could not be detected after 1.5 h of heating.

After 6 h of heating at 180 °C, approximately 58% of carotenoids were lost from olive oil and 50% from avocado oil, but in the rice bran oil no loss was observed.

Although there were significant losses of both pigments in avocado oil, levels remained significantly higher in this oil, when compared to the others at all heating time points.

Procida et al.³² evaluated the influence of chemical composition on the development of volatile compounds during frying in olive oil. They found a negative effect of temperature on these pigments (chlorophylls and carotenoids), confirming that when a high temperature was applied, these pigments were damaged.

Carotenoids are tetraterpenoid compounds, which can have 3 to 15 double bonds in conjugation. Isomerization and oxidation are the two major carotenoid alterations that occur during thermal processing. During heating, it is assumed that part of the β -carotene fraction is converted into isomers and the other part is involved in oxidative degradation reactions³³.

Carotenoids act as biological antioxidants and seem to play an important role in human health by protecting cells and tissues from damaging effects, such as those related to cancer³. This justifies the importance of the presence and maintenance of these compounds in foods.

The chlorophyll molecule consists of a tetrapyrrolic structure with Mg^{2+} in the core (porphyrin ring), besides

an additional fifth isocyclic ring and side chains attached, of which an important one is called phytol. The proposed degradation mechanism for chlorophyll involves reactions that affect the porphyrin ring and the structure of the isocyclic ring of pheophytin³⁴.

Antioxidant Capacity – ABTS

There is an increased interest in studying the antioxidant properties of natural compounds and food components³⁵. It is advisable to control these properties during food processing, in order to improve the nutritional quality and stability of the food products. Thus, the evaluation and effectiveness of antioxidant activity are relevant factors for predicting the usefulness of any oil³⁶.

The analysis of antioxidant potential is considered a diagnostic challenge because the current methods can analyze only aqueous samples, and adaptations in the assay are required to obtain better results with lipophilic samples. However, Koidis et al.³⁷ suggested that the antioxidant activity of olive oil is generally determined by the ABTS method or by the estimation of the DPPH radical, which are based on the ability to protect against oxidation by peroxy radicals.

It was found that the antioxidant capacity of all lipid samples was negatively affected by heating time. Numerically, the differences in values were not very expressive; however, in general, rice oil showed a significant advantage ($p \leq 0.05$). Comparing the initial condition (unheated sample) with the final condition (6 h of heating), there was a 60% reduction in the antioxidant capacity of rice oil, 65% in avocado oil and 73% in olive oil.

Olive oil was the only sample that had significant change in antioxidant capacity with the heating time.

This small advantage of rice oil may have been a consequence of containing γ -oryzanol, a compound with recognized antioxidant activity. Also, the disadvantage of olive oil may be due to the greater losses of carotenoids that it suffered.

According to Wang et al.³⁸ rice bran oil has higher antioxidant activity than other oils, and this activity increases markedly with the concentration of γ -oryzanol, due to the high antioxidant potential of this compound.

Phenolic compounds

Phenolic compounds are an important class of natural products that prevent oxidative processes in plants, by exerting antioxidant activity against free radicals produced during photosynthesis³⁵.

In general, some observed oscillations in the phenolic compounds content may be associated with the experimental error of the technique. However, the content of phenolic compounds was maintained in the rice bran and olive oils, even after 6 h of heating at 180 °C. In avocado oil, there was a significant reduction of these compounds with the increase in heating time.

Olive oil and avocado oil are known for containing significant amounts of phenolic compounds; in fact, in the

unheated sample values were relatively close and higher than in rice oil. However, the stability of these compounds was lower in avocado oil, probably due to the chemical characteristics of the phenolic compounds present in each sample and/or the protection provided by other minority compounds in the products.

There was no relationship between the content of phenolic compounds and antioxidant capacity, since olive oil showed higher levels of phenolic compounds during heating and, on the other hand, less antioxidant capacity.

There is a discrepancy regarding the content of phenolic compounds of olive oil in the literature (ranging from 5 to 1000 mg.kg⁻¹), but values are generally between 100 and 300 mg.kg⁻¹. Cultivation, the extraction system, processing conditions, packaging, distribution, and storage are critical factors that may affect the final phenolic content of the product³⁹.

Quiles et al.⁴⁰ reported that the reduction in phenolic content is a consequence of the frying process. This is probably because of the thermal destruction of these compounds or because of their use in the protection of vegetable oils against oxidation.

CONCLUSIONS

It was established that the heating of oils had an impact on the results of most of the evaluated physicochemical parameters, thus influencing product stability.

The negative effect was more intense in rice bran oil, reflecting higher levels of oxidation compounds, both primary and secondary, as well as greater loss of unsaturation and chlorophyll content. On the other hand, antioxidant capacity was least affected by heating. Avocado and olive oils were similar in many evaluated parameters. Significantly higher levels of pigments were found in avocado oil compared to the other oil samples, at all heating time points. While for olive oil, regarding the phenolic content, a higher level was seen compared to other oils.

It should be noted that avocado and olive oils stood out in relation to rice bran oil when heated to 180 °C, showing higher thermal stability than the refined oil.

This work contributes with information about the thermal stability of avocado oil, evidencing its similarity with olive oil and demonstrates that both oils are fairly stable at the normal temperature of frying.

Founding Source. CNPq for funding (443288/2014-3).

Acknowledgements. The authors thank the avocado producer Mr. José Carlos Gonçalves for providing avocado oil samples.

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