



RESEARCH PAPER

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Antioxidant effect of *Urtica dioica* on the stability of rapeseed oil during deep frying of french fries

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Abstract

Antioxidant capacity of *Urtica dioica* extract used in dietology practice was determined by DPPH free radical method. Partially hydrophilic phenolic compounds are the most active compounds in plants. Therefore methanol was used as the extraction agent. The total phenolics content were also measured and a strong correlation between these two variables was found. It is important to study about the use of natural antioxidants as alternatives to synthetic ones due to the possibility of carcinogenic effects of synthetic antioxidants. The aim of this study was to determine the antioxidant activity of methanolic extract of *Urtica dioica* leaves and comparing its antioxidant effect at levels of 100 and 800 ppm with synthetic antioxidant TBHQ at level of 100 ppm on the oxidative stability of rapeseed oil during deep frying of French fries. Results showed amount of phenolic compounds extracted by methanol were 87.127 ± 6.096 mg gallic acid equivalent/g dry sample and antioxidant capacity was 0.303 ± 0.025 mg. Results of peroxide value showed oil containing 100 ppm TBHQ had the lowest peroxide value (1.794 meq O₂/Kg oil) after 96 h of deep frying. Results of acid, iodine and anisidine values indicated TBHQ has been more effective on stability of rapeseed oil after 48 h of deep frying. According to the results of sensory evaluation, samples fried in oil containing 100 ppm extract had the highest score throughout 48 h of frying. It is necessary to investigate higher concentrations of *Urtica dioica* leaves extract and compare with other synthetic antioxidants.

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Introduction

Frying is the most favorable methods of cooking in the world, both industrial as well as domestic. The fried productions have sensory features including taste, texture and appearance which lead to enjoyment by the consumer. Also, this method significantly reduces the cooking time (Casal *et al.*, 2010). One of the main reactions of the deterioration during the processing and storage of fried foods is lipid oxidation which leads to the spread of bad taste and food is less acceptable (Tabee *et al.*, 2008). Hence the quality of frying oil is important because of its absorption to food during processing and its impact on the quality of the final product (Stenson and Min, 2000). The oil manufacturers, generally, add antioxidants to reduce the undesirable changes and increased shelf life of fried products during frying and storage (Jaswir *et al.*, 2000). The Toxic and carcinogenic effects of synthetic antioxidants on human health has led to increased use of natural antioxidants in the frying oil (Inanç and Maskan, 2012).

The *Urtica dioica* (Nettle) is one of the plants that need further evaluation because of its antioxidant activity and phenolic compounds (Inanç and Maskan, 2012). *Urtica dioica* a herbaceous perennial flowering plant, is a member of the *Urticaceae* family. Plants belonging to the *Urticaceae* family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties (Kukric *et al.*, 2012). Nettle has a long history as a medicinal plant and food (Guil-Guerrero *et al.*, 2003) and has been used to treat stomach ache in Turkish traditional medication. Additionally, this plant is also used for the treatment of rheumatic pain, colds, cough, chest pain and liver insufficiency (Gulçin *et al.*, 2004). Analysis the methanol extract of *Urtica dioica* by using the chromatography showed that vanillic acid, homo-vanillic acid, 2-hydroxy Syamic acid, 4-hydroxy Syamic acid and frolic acid were isolated as effective antioxidant compounds (Fiamegos *et al.*, 2004). Dall`Acqua *et al.*, (2008) were evaluated the antioxidant capacity of methanolic solution of *Urtica dioica* leaves with 2,2-diphenyl-1-

picrylhydrazyl (DPPH) method and reported that the antioxidant activity was $419 \pm 10 \mu\text{g/ml}$. The total phenolic content was found to be $0.35 \pm 0.02 \text{ mg/l GAE}$. Numeral studies have been done about other medical plants. Nor *et al.*, (2009) expressed that *Curcuma-longa* leaf extract concentrations of 0.2% compared to 0.02% Butylated hydroxytoluene (BHT) was able to significantly ($P < 0.05$) reduce the oxidation of palm olein oil during deep frying at 180°C for 40 hours and based on the sensory evaluation, French fries were not significantly different ($P < 0.05$) from each other. Cheung *et al.*, (2003) has compared the antioxidant effect of edible mushroom extract with synthetic antioxidant *tert*-Butylhydroquinone (TBHQ) and concluded that TBHQ at a concentration of 2 ppm had the higher antioxidant effect of than mushroom extract at 20 ppm.

Considering surveyed and reviewed literatures most of the researches accomplished in antioxidant effect of *Urtica dioica* which shows the necessity of doing an investigation on *Urtica dioica* leaves effect on the stability of oil during frying. The objective of this study was to investigate the antioxidative properties of *Urtica dioica* leaves extract in rapeseed oil. The antioxidant activity of plant extract was compared with that of TBHQ during 48 hours of deep frying at 180°C . Also, organoleptic quality of French fries was evaluated.

Materials and methods

Materials

Urtica dioica prepared from Research Center of Agriculture and Natural Resources of Tabriz. The leaves were separated from the stems and dried in the shade at the room temperature using air and was turned into powder using a mill and passing through a sieve. Fresh potatoes of the same variety (Agrida var.) purchased from local market, manually peeled and grated manually to slice-potatoes of same size. Refined, bleached and deodorized (RBD) rapeseed oil used in this study was obtained from local refinery in Tabriz. TBHQ was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All solvents and other

chemicals of analytical grade were purchased from Merck Co. (Germany).

Sample preparation

125g of dried powder was weighed and extracted with 1250ml of methanol for 24h at the room temperature in the dark. The obtained extract was filtered using Whatman No.1 filter paper and dried in rotary evaporator and kept in airtight container at 4°C for further analysis (Lin *et al.*, 1999).

Frying experiment

The antioxidant treatments were:

Control: rapeseed oil without antioxidant

U₁₀₀: rapeseed oil with *Urtica dioica* extract of 100ppm

U₈₀₀: rapeseed oil with *Urtica dioica* extract of 800ppm

T₁₀₀: rapeseed oil with *Urtica dioica* extract of 100ppm TBHQ.

Fresh potatoes were peeled and sliced to a thickness of 2 mm using a hand slicer. They were kept submerged in distilled water at room temperature. They were then slightly dried with tissue paper before weighing into 100 g batch for frying. Rapeseed oil (10 Kg) was put into a stainless steel electric deep-fat fryer (Fritaurus Professional 4, Oliana, Spain). The oil was heated at 180 ± 2°C. Then, the heated oil was kept at 180 ± 2°C for 8 hours. Temperature was monitored with a digital thermometer (IKA Labrotechnik ETC 1, Germany) consistently. After 8 h, 100 g of raw sliced potatoes were put in this oil and fried for 4 min. Then oil samples were collected in amber bottles. The next day, the same oil that was heated 8 h, kept at 180 ± 2°C until 16 h. After 16 h new batch of raw potatoes were fried. The frying experiment was carried out for 8 h per a day. This is equivalent to 48 h frying at 6 consecutive days and 24 batch of French fries. At the end of every day, the fryer switched off and oil samples were collected in amber bottles every 8 h of frying and kept at 4°C for further analysis. Analysis of oil was carried out immediately after the frying experiment. Fresh oil was not added to the fryer. Since the peroxide value is

one of the important determination parameters of the oil oxidative stability, just in this case, frying parameter continued to 96 hours. Fried potatoes were collected every 8 h of frying and packed in aluminum laminate bags and stored at 4°C for 30 d for sensory analysis.

Measurement of total phenolics content

The total phenolics content in the extract examined by the colorimetric method by Folin-Ciocalteu and according to Cemeroglu, (2010). The absorbance was measured at 760 nm using a spectrophotometer (Unico UV- 2100, USA). The concentration was calculated using gallic acid as a standard, and the results were expressed as gallic acid equivalents per gramme of extract.

Measurement of antioxidant activity

The free radical-scavenging activity was assayed, based on the reduction of DPPH radicals in methanol, which causes an absorbance drop at 517 nm. The extract's activity against the DPPH radical was evaluated using method of Cemeroglu (2010). 1mM solution of DPPH* in methanol was prepared. Then, 600 µl of this solution was added to 20,40,60,80 and 100 µl of methanolic extract of *Urtica dioica* leaves and was brought to 6ml with methanol. Methanol (5.4 ml) with DPPH solution (600 µl) was used as blank. These solution mixtures were kept in dark at room temperature for 15 min and the absorbance was measured at 517 nm using a spectrophotometer. Radical scavenging activity was expressed as the percentage inhibition using the formula given below:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Analysis of oil quality

Chemical changes were conducted in triplicate by measuring parameters such as peroxide value (PV), iodine value (IV), acid value (AV) and anisidine value (AnV). All tests based on The American Oil Chemist's Society Official Methods (AOCS).

Sensory evaluation

The sensory analysis was done on 8, 16, 24, 32, 40 and 48 h of frying by using a five-point hedonic scale. 10 untrained panelists randomly were selected from students and laboratory staffs of Faculty of Food Science and Technology of Islamic Azad University of Tabriz. Training is done in order to make students familiar with the concepts of sensory evaluation. Each sample was coded with a three digit random number. Panelists were required to evaluate the sensory attributes of French fries, including color, taste, odor and overall acceptability, by giving a score ranging from 1 (very poor) to 5 (very good).

Statistical analysis

All analysis was conducted triplicate. The obtained data from frying experiment at different times were analyzed by repeated measurement. The obtained

data from sensory evaluation was carried out in a (4×6) factorial scheme and was analyzed by completely randomized block design. The statistical analyses of data were performed using the SAS software (version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). Duncan's multiple-range tests and Tukey tests were used to compare the difference among mean values at the significant level of 0.05 ($P < 0.05$).

Results and discussion

Total phenolics content and antioxidant activity

The results showed that the amount of phenolic compounds extracted by methanol is 87.127 ± 6.096 mg gallic acid equivalents/g extract and antioxidant activity of *Urtica dioica* is obtained 0.303 ± 0.025 mg.

Table 1. The results of interaction effect of antioxidant and frying time on the score of color.

Time (h)	8	16	24	32	40	48
Antioxidant type						
Control	1.1 ± 0.316^g	3.3 ± 0.48^{abc}	2.4 ± 0.699^{def}	3.7 ± 0.948^{ab}	2.60 ± 0.516^{cdef}	3.2 ± 0.421^{abcd}
U ₁₀₀	3.2 ± 0.788^{abcd}	2 ± 0.471^f	1.9 ± 0.567^f	2 ± 0.816^f	3.7 ± 0.647^{ab}	3.9 ± 1.28^a
U ₈₀₀	2 ± 0.816^f	1.9 ± 1.1^f	3.2 ± 0.421^{abcd}	2.2 ± 1.22^{ef}	3.7 ± 0.483^{ab}	3.6 ± 0.966^{ab}
T ₁₀₀	3 ± 0.94^{bcd}	3.7 ± 0.647^{ab}	2.4 ± 1.07^{def}	3.4 ± 0.699^{abc}	3.3 ± 0.823^{abc}	2.9 ± 0.875^{bcde}

Table 2. The results of interaction effect of antioxidant and frying time on the score of odor.

Time (h)	8	16	24	32	40	48
Antioxidant type						
Control	1.8 ± 0.918^e	2.5 ± 0.707^{abcde}	2.6 ± 0.966^{abcde}	2.6 ± 0.966^{abcde}	2.8 ± 0.632^{abcde}	2.3 ± 0.948^{bcde}
U ₁₀₀	2.4 ± 1.349^{abcde}	2.3 ± 0.482^{bcde}	2.1 ± 1.1^{cde}	1.9 ± 1.28^{ed}	2.8 ± 0.632^{abcde}	3.4 ± 1.26^a
U ₈₀₀	2.2 ± 0.918^{bcde}	2.4 ± 0.843^{abcde}	2.7 ± 0.948^{abcde}	1.8 ± 0.788^e	3.2 ± 1.135^{ab}	2.9 ± 0.994^{abcd}
T ₁₀₀	2.7 ± 1.059^{abcde}	2.8 ± 0.632^{abcde}	2.9 ± 0.737^{abcd}	2.9 ± 0.737^{abcd}	3 ± 0.816^{abcde}	2.7 ± 0.948^{abcde}

Peroxide value (PV)

Figure 1. shows the comparison of the *Urtica dioica* extracts with synthetic antioxidants in terms of peroxide value during 96 h frying of rapeseed oil. In all treatments, at the first 40 h, the peroxide value increases occurred at lower speeds, but after that, the pace was very high in the peroxide value of control, which indicates high potential for spoilage in this treatment. But peroxide value of T₁₀₀ is less than the other treatments. Also, in comparing two treatments,

U₁₀₀ and U₈₀₀, it is observed that with increasing concentration from 100 ppm to 800 ppm, the peroxide value was decreased significantly ($P < 0.01$) from 99.1 to 89.1 meqO₂/Kg. which is consistent with the findings of Nor *et al.*, (2008) and also Gharekhani *et al.*, (2010). In agreement to our results, a previous study by Nor *et al.*, (2008) on *Pandanus amaryllifolius* leaf extract in deep frying studies showed that high concentration of extract increased the antioxidant activity, accordingly reduced peroxide

value. Similarly, Gharekhani *et al.*, (2010) also reported significant differences among different concentration of extract and antioxidant activity in *Urtica dioica* extract.

Iodine value (IV)

Figure 2. shows the comparison of *Urtica dioica* extracts with the synthetic antioxidant in the terms of iodine value during 48 h frying of rapeseed oil. At the first 8 h, iodine value descending of all samples was almost the same, as heating oil during deep frying lead to oxidation and influences the double bonds of unsaturated fatty acids, and results in the iodine value

reduction. Reduce in iodine value of oil after frying, indicates its more oxidized state (Jaswir *et al.*, 2005). Changes in iodine value of control indicate that the rate of oxidation of unsaturated fatty acids decreases in the presence of the antioxidant. Changes in U_{100} , U_{800} and T_{100} are almost identical at all hours and at the end of 48 h, T_{100} had the highest iodine value than the others. Nor *et al.*, (2008) reported that *Pandanus amaryllifolius* leaf extract and BHT are both prevent the reduction of iodine value in palm olein oil better than the control, but after 24 h, BHT showed a better ability to protect the oil which is in agreement with our results.

Table 3. The results of interaction effect of antioxidant and frying time on the score of taste.

Time (h)	8	16	24	32	40	48
Antioxidant type						
Control	1.6±0.843 ^f	2.6±1.349 ^{abcdef}	2.5±0.849 ^{abcdef}	2.6±0.966 ^{abcdef}	2.6±1.07 ^{abcdef}	2.5±0.707 ^{abcdef}
U_{100}	2.5±0.707 ^{abcdef}	2.1±0.944 ^{def}	2.3±0.948 ^{bcdef}	2.3±1.251 ^{bcdef}	3.3±0.823 ^{ab}	3.4±1.173 ^a
U_{800}	1.6±0.699 ^{abcdef}	2.6±0.843 ^{abcdef}	2.9±0.567 ^{abcde}	1.9±0.875 ^{ef}	3.2±0.918 ^{abc}	2.7±1.059 ^{abcde}
T_{100}	2.6±0.966 ^{abcdef}	2.8±0.788 ^{abcde}	2.2±0.788 ^{cdef}	2.6±1.032 ^{abcde}	3.4±0.966 ^a	3.1±0.875 ^{abcd}

Table 4. The results of interaction effect of antioxidant and frying time on the score of overall acceptability.

Time (h)	8	16	24	32	40	48
Antioxidant type						
Control	1.4±0.699 ^s	2.7±0.823 ^{cdef}	2.4±0.699 ^{def}	3.3±0.948 ^{abcd}	3.2±1.032 ^{abcde}	2.7±0.647 ^{cdef}
U_{100}	2.8±0.788 ^{bcde}	2.4±0.843 ^{def}	2.4±0.516 ^{def}	2.2±1.316 ^{efg}	3.3±0.923 ^{abcd}	3.5±1.51 ^{abc}
U_{800}	1.8±0.788 ^{gf}	2.7±1.159 ^{cdef}	3.3±1.05 ^{abcd}	2.2±0.918 ^{efg}	3.8±1.135 ^{ab}	3.3±1.251 ^{abcd}
T_{100}	3.1±1.1 ^{abcde}	3.9±1.1 ^a	3±0.471 ^{abcde}	3.2±0.788 ^{abcde}	3.3±0.674 ^{cdef}	2.8±0.632 ^{bcde}

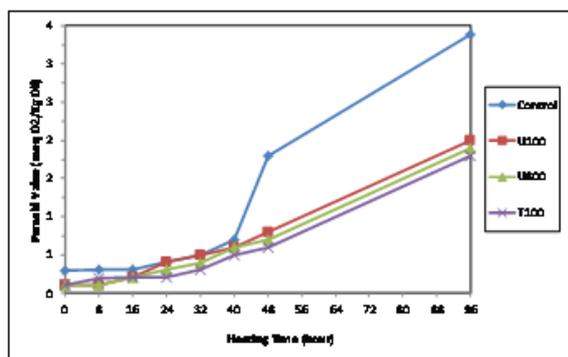


Fig. 1. Comparison of *Urtica dioica* extracts with the synthetic antioxidant in the terms of peroxide value during 96 h frying of rapeseed oil.

Acid value (AV)

The large amounts of free fatty acids are indicative of

degradation of oil during frying and development of spoilage in fried foods. A part of the increase in the acid value is attributed to hydrolysis of triglycerides and the other carbonyl groups present in the polymeric or oxidative products (Jaswir *et al.*, 2005). At the first 8 h of frying, increase in the acid value in control, T_{100} and U_{100} was almost the same. U_{800} was more effective than other samples from 0 to 32 h of frying. This indicated that U_{800} was able to retard oxidation. Comparing the effect of extracts (U_{100} and U_{800}) it is also observed that the increase in free fatty acids in U_{800} was less than in U_{100} and this indicates that with increasing concentration of extract, its impact also has increased (Figure 3). Nor *et al.*, (2009) expressed that *Curcuma longa* extract was

better than BHT could reduce the free fatty acids after 24 h deep frying of palm olein oil. Che Man and Tan, (1999) concluded that after seven consecutive days of deep frying of palm olein oil, the amount of free fatty acids in the control > BHT > sage extract > BHA \approx rosemary extract. The results of present study correspond with the findings of other investigators.

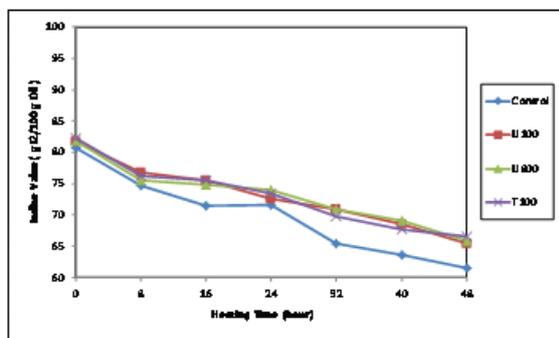


Fig. 2. Comparison of *Urtica dioica* extracts with the synthetic antioxidant in the terms of iodine value during 48 h frying of rapeseed oil.

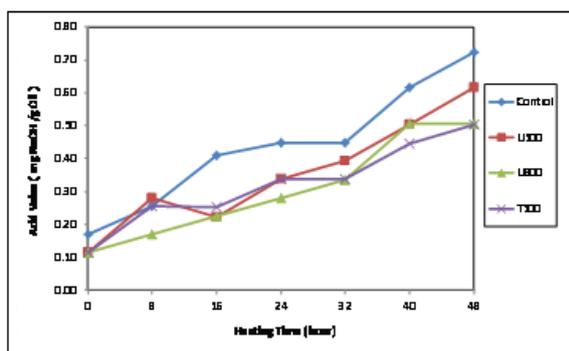


Fig. 3. Comparison of *Urtica dioica* extracts with the synthetic antioxidant in the terms of acid value during 48 h frying of rapeseed oil.

Anisidine value (AnV)

ρ -Anisidine is the index of volatile and nonvolatile aldehydic compounds present in the oils and fats, mostly 2-enals and 2,4-dienals. The main factor contributing to increase ρ -Anisidine during oil heating is the aldehydes formation (Kalantzakis and Blekas, 2006). At the first 8 h, increase of anisidine value was almost identical for all samples, but then T₁₀₀ has shown its effect and could significantly ($P < 0.01$) inhibit the aldehydes increase. Comparing the effect of the extracts it is also observed that both U₁₀₀ and U₈₀₀ from 0 to 32 h had almost the same effect but after a time of 32 h, U₁₀₀ has shown less

increase (Figure 4). Chirinos *et al.*, (2011) reported that the anisidine value for all the samples increased with time of frying, but for *Clinopodium bolivianum* leaves extract was less than TBHQ which indicates that the formation of carbonyl groups was lower in soybean oil containing this extract. Shelbaya *et al.*, (2011) demonstrated that the formation of secondary compounds was increased with time of frying in all samples and it was more for control than the other samples. Also, 0.3% concentration of petroleum ether extract of *Malva sylvestris* has the lowest anisidine value, even less than BHT.

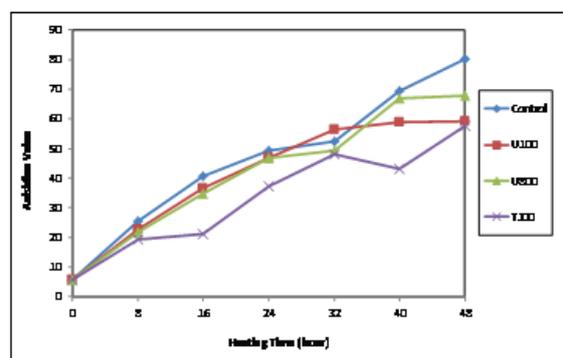


Fig. 4. Comparison of *Urtica dioica* extracts with the synthetic antioxidant in the terms of anisidine value during 48 h frying of rapeseed oil.

Sensory evaluation of French fries

The results of interaction effect of antioxidants and frying time on the score of color, odor, taste and overall acceptability of French fries are given in the tables 1, 2, 3 and 4. The sensory results showed that there were no significantly differences ($P < 0.05$) in organoleptic qualities of samples fried in the oil containing synthetic and natural antioxidants. Samples fried in control were assessed as the most unfavorable French fries by panelists, which seems due to the breakdown of triglycerides, oil had unpleasant taste and odor. Color was also darker comparing the other samples. Lalas and Dourtoglou, (2003) stated that potato chips fried in the oil without rosemary extract had a pungent taste comparing to the oil with rosemary extract, due to the oxidation of absorbed oil in potato chips. Evaluation of all attributes showed that samples fried in U₁₀₀ were the most favorable French fries evaluated by panelists and had the highest score throughout 48 h of frying.

According to Table 4. it can be observed that the scores increased significantly ($P < 0.05$) with the frying time for the samples fried in U_{100} , unlike the samples fried in T_{100} , where the scores decreased significantly ($P < 0.05$) with the frying time. This indicates that the oil contains 100 ppm *Urtica dioica* extract could maintain its effect after 48 h of frying but T_{100} lost its effect within the time. Therefore, *Urtica dioica* extract can maintain the quality of fried products.

Che Man and Tan, (1999) reported that sensory evaluations of potato chips fried in palm olein containing BHT, Butylated hydroxyanisole (BHA), rosemary extract and sage extract, in terms of taste, odor, texture and overall acceptability did not show significant ($P < 0.05$) differences between all treatments. Nor *et al.*, (2009) reported that fried samples in oil containing BHT and *curcuma longa* extract had no significant ($P < 0.05$) difference in the terms of flavor, color and crispiness. The sensory evaluation results showed that *curcuma longa* extracts could preserve the quality of fried products. The results of this study in accordance with results of other investigators and herbal extracts used in the oils are able to improve the organoleptic quality of the fried products better than synthetic antioxidants.

Conclusion

The results of this study demonstrated that synthetic antioxidant TBHQ has been more effective to improve the oxidative stability of rapeseed oil during 48 hours of deep frying which could be attributed to the low concentration of the *Urtica dioica* extract used in this study and high performance of TBHQ. Due to the lower efficiency of the *Urtica dioica* extract, based on the results of this study, it is necessary to investigate the higher concentrations of *Urtica dioica* extract and comparing with other synthetic antioxidants. Based on the sensory evaluation, samples fried in the oil containing 100 ppm of *Urtica dioica* extract was selected as the most desirable French fries throughout 48 hours deep frying. Although the natural sensory attributes of *Urtica dioica* extract, is not compatible with those of French fries but at low usage level (100 ppm) could be pleasant.

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