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Characterisation and use of single and double low temperature dry-fractionated olein and stearin from virgin olive oil

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Received: 15 July 2013 / Accepted: 23 November 2013

RESEARCH ARTICLE

Abstract

Single and double-fractionated olein and stearin were obtained by successive cooling of the virgin olive oil at 10 and 6 °C. Characteristics of the obtained fractions were compared with those of the virgin parent oil. Single-fractionated stearin (first stearin) constituted 65% (w/w) of the virgin oil, while double-fractionated stearin (second stearin) constituted 55% (w/w) of the single-fractionated olein (first olein). The fractionation process resulted in minor changes in the peak temperature and enthalpy change of the differential scanning calorimetry thermograms, and in minor changes in the physical, chemical sensory attributes of the oil fractions. The virgin oil and the stearin fractions were significantly ($P \leq 0.05$) more stable to oxidation as evaluated by the Rancimat method than the olein fractions. Mayonnaise and Italian salad dressing prepared from the double fractionated stearin had significantly ($P \leq 0.05$) superior consistency, smoothness and overall acceptability to those produced from the other fractions.

Keywords: differential scanning calorimetry, fractionation, olive oil, salad dressing

1. Introduction

Olive oil is one of the most valued edible oils; it is used by people around the Mediterranean and other parts of the world in both cold and hot applications. However, it gets cloudy when stored in the refrigerator (www.oliveoilsource.com/page/freezing-olive-oil) which makes it less appealing to consumers who like it to be as clear as possible. Mayonnaise prepared from olive oil has a superior nutritional value yet with a rather harsh texture; a problem usually overcome by using winterised olive oil (Firestone 2005). Another problem related to olive oil, based mayonnaise is the separation of the oil phase when the product is taken out of the refrigerator; a phenomenon attributed to 'changes in crystallisation properties of the oil' (Fomuso *et al*., 2001).

Cold temperature, dry-fractionation of oils to stearin and olein (Kellens *et al*., 2007) is a standard practice in the fats and oils industry. It is known to help processors develop new uses for the given oil, as it imparts some new physical properties to the obtained fractions without drastically changing their chemical or nutritional characteristics. It is a process by which the physicochemical properties of the oil are manipulated through selectively separating the triglycerides according to their crystallisation behavior at different temperatures. The process usually consists of two stages: crystallisation by cooling and physical separation by filtration, centrifugation or other physical means (Kellens, 1998). The first cold temperature fractionation of olive oil was reported by Holde and Stange in 1901 (Kellens *et al*., 2007) who separated small solid crystals from olive oil by cooling it in ether at -40 °C. Cold temperature, dry single and double fractionated palm oils are popular products worldwide (Ong and Goh, 2002); their production resulted in increasing the market share and uses of this tropical oil as it became possible to use it in a wider range of climates and applications. In case of olive oil, winterisation which is a form of fractionation, helps remove waxes and high melting triacylglycerols. The process is based on cooling the oil 'maturing' to 5-8 °C in order to increase the size of crystals, and adding 5% water to separate the stearin fraction (Boskou, 2002).

Despite its high price and use mainly in cold applications, the increased production of olive oil coupled with the increased consumer demand for health and functional foods makes it quite viable to search for new applications and develop new olive oil-based products. Hence, the objective of this work is to obtain olive oil fractions by cold temperature, dry-fractionation, study the physical and chemical characteristics of the obtained fractions and use them in the preparation of salad dressing and mayonnaise.

2. Materials and methods

Olive oil samples

Two 10-liter cans of Jordanian virgin olive oil (2 replicates), cold pressed from rain-fed Nabali olive cultivar grown in the same area, were purchased separately at two-week intervals from a local olive press during the crop season of 2010. Both replicates were stored at room temperature (14-18 °C) in winter for about two months, then each one was kept in a household refrigerator for one week at 10 °C. The oil from each can was then separated (single fractionated) into first olein and stearin by filtration through two layers of gauze (grade 50, cotton cheesecloth gauze, 24×28 threads/645.16 mm^2). The so-obtained olein (first olein) fraction was cooled further to 6 °C and separated the same way (double fractionated) into second olein and stearin. In both cases, each fraction was weighed and its quantity expressed as %w/w of the original virgin oil. Thus, two olein (first and second) and two stearin (first and second) fractions were obtained in addition to the virgin oil.

Physical and chemical characterisation

Specific gravity, refractive index, iodine number and saponification number were performed according to the official methods of the Association of Official Analytical Chemists (AOAC, 1995). Total phenolic compounds were estimated according to the method described by Capannesi *et al*. (2000) and the results were expressed as µg Gallic acid/g sample. All tests were run in duplicates.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was run on all fractions in duplicates using DSC instrument model NETZSCH-DSC 204 F1-Phoenix (NETZSCH-Gerätebau GmbH, Selb, Germany) supplied with Proteus software (Labcenter Electronics Ltd., Grassington, UK) and calibrated according to the instructions of the manufacturer using indium, bismuth, mercury, zinc and tin. The samples (2-3 mg) were weighed into aluminium pans to the nearest 0.001 mg using a microbalance (RADWAG MAX5; Bracka, Poland). Samples were heated from -90 to +90 °C at a rate of 10 °C/min with a nitrogen purging gas flow of 30 ml/min and a protective gas flow of 70 ml/min. All samples were run in duplicates. Onset temperature, peak temperature and enthalpy change (ΔH) expressed as J/g for the major peak of each sample were recorded.

Oxidative stability of the oil

Oxidative stability of the olive oil samples was evaluated using a Rancimat instrument (Metrohm-743; Metrohm, Herisau, Switzerland) supplied with Metrodata 743 PC software (version 1.0; Metrohm) based on the AOCS method (Firestone, 1992).The tests were carried out in duplicates each weighing 3 g of oil, each was placed on one of the two sides of the thermostatic block at 100 °C, with an air flow speed of 20l/h. New glassware (reaction vessels with air inlet tubes) was used for each run to avoid any contamination or interference with the results. Measuring vessels, electrodes, and connecting tubes were cleaned several times with a detergent then rinsed with acetone and distilled water (Farhoosh, 2007; Frega *et al*., 1999) except the measuring vessel which was cleaned with ethanol instead of acetone. All parts were blown out with air after cleaning and before the experiment was started. Volatiles were collected in the measuring vessel under 60 ml of distilled water (Savage *et al*., 1997; Velasco *et al*., 2004). The data collected was transformed by the software into induction times in h of the peroxidation curves.

Fatty acid analysis

Fatty acid methyl esters were prepared according to the method described by Tsaknis *et al*.(1999) whereby a 50 mg. sample of the oil was dissolved in 2 ml hexane (GC grade) and mixed thoroughly with a vortex for 1 min. A 200 µl of 2 M KOH in MeOH was added and mixed for 30 sec until the solution was clear, then 200 µl acetic acid was added and mixed for 30 sec. The so-obtained methyl esters were analysed using a GLC instrument (model 2010; Shimatzu Inc., Koyoto, Japan) supplied with a capillary GLC TR-FAME column (Thermo Scientific, Bellefonte, PA, USA; crossbond 70% cyanopropyl polysilphenylene-siloxane, 60 m, 0.25 mm ID, 0.25 µm film). Injection was carried out using a 1 µl hexane layer through the injection port. Analysis was carried out under programmed conditions as follows: column oven temperature was set at 165 °C for 8 min then increased to 180 °C at a rate of 1 °C/ min and kept for 1 min then increased to 220 °C at a rate of 3 °C/min and kept at this temperature for 10 min. Injector temperature was 240 °C while flame ionisation temperature was 260 °C with a flow rate of 0.8 ml/ min helium was used as a carrier gas with a split ratio of 80. The fatty acids were identified using a fatty acid methyl ester standard of 12-24 carbons (Sigma Aldrich, St. Louis, MO, USA) and quantified using a digital integrator.

Preparation of mayonnaise and Italian salad dressing

Mayonnaise consisted of the following ingredients (w/w): egg powder (2.5%); olive oil (68%); vinegar (24% acetic acid) (1.3%); salt (1.3%); sugar (2.8%); water (24%); and xanthan gum (0.1%). Mayonnaise was prepared in a Hamilton Beach mixer (model 936-1 drink mixer; Southern Pines, NC, USA) by vigorously blending the dry ingredients with water and vinegar while oil was gradually mixed in to create an emulsion. The Italian salad dressing consisted of olive oil (60%), cider vinegar (20%), water (10%), garlic powder (0.5%), sugar (4%), salt (3%), xanthan gum (0.5%), dry thyme (1%) and fresh peppermint leaves (1%). The dry ingredients were mixed vigorously with the water and vinegar for 2 min using the same mixer above, then the peppermint was added and mixed for 30 sec, then the oil was added and mixed for 1 min until an emulsion was formed. Samples of the mayonnaise and dressing prepared from the virgin oil its fractions were filled in 100 ml glass jars and kept in the refrigerator.

Sensory evaluation

Oil samples were evaluated by an unofficial 5-member taste panel (official panel requires 8-12 panellists) all of which are members of the Jordan Olive Oil National Team and certified by the International Olive Oil Council (IOOC, 1996). The evaluation procedure was carried out following the protocol set up by the council (IOOC, 1996) by which the intensities of perception of both defects (fusty, musty, acid-sour, metallic and rancid) and positive attributes (fruity, bitter and pungent) were evaluated on a scale of 1-10 (10 being highest). Mayonnaise and salad dressing samples were evaluated by using the triangle test (Meilgaard *et al*., 1999).

A 5-member panel composed of graduate students and employees from the department of Nutrition and Food Technology/University of Jordan were trained on the evaluation of mayonnaises and Italian salad dressing. Panellists were asked to identify the odd sample among the three samples presented in a random order (two identical and one different) and prepared from the virgin and fractionated oils. The two panels included members of the two sexes ranging in age between 24-30 years and are habitual consumers of olive oil, salad dressing and mayonnaise.

Statistical analysis

Data for Rancimat induction times and phenolic compounds were analysed using a SAS package (SAS Institute, 1996). Analysis of variance (ANOVA) and least significant difference (LSD) tests were performed. A randomised complete block design was followed with blocking on replicates (Steel *et al*., 1996). Results of the sensory evaluation of the mayonnaise and Italian salad dressing were interpreted by referring to the table of critical numbers of correct responses in a triangle test (Meilgaard *et al*., 1999).

3. Results and discussion

Fraction characterisation

Table 1 shows the percentage of each fraction obtained by the dry fractionation process. At 10 °C the stearin fraction (first stearin) constituted 35% (w/w) of the total quantity of the virgin oil, while the olein fraction (first olein) constituted 65% of it. Upon further fractionation of the first olein by cooling at 6 °C, 55% of this fraction (35% of the virgin oil) was obtained as stearin (second stearin) and 45% as second olein (30% of the virgin oil). The specific gravity of the virgin oil (0.910-0.916 at 20 °C/20 °C water) is in compliance with the standard set up by the Codex Alimentarius Commission (2003). However, the fractions have specific gravity values (0.9086-9090) lower than that of the virgin oil sample (0.914) or the value specified in CODEX Alimentarius Standard (2003) for virgin olive oil, yet they fall within the range (0.8-0.92) reported by some sources for the same grade of oil (www.csgnetwork.com/specificgravliqtable.html).

The refractive index was the same for all samples (1.4677) which is well around the values reported for virgin olive oil

Table 1. Some physical and chemical characteristics of the virgin olive oil and its fractions obtained by single and double fractionation at 10 and 6 °C.

Sample	% of virgin oil (w/w)	Specific gravity (g/cc at 20 °C)	Refractive index	Iodine number	Saponification number
Virgin oil	100	0.9140	1.467	88.5	195.1
First stearin	35	0.9086	1.467	79.8	196.1
First olein	65	0.9089	1.467	86.3	190.0
Second stearin[1]	35	0.9090	1.467	82.1	193.5
Second olein[1]	30	0.9088	1.467	92.5	193.1

[1] Obtained by cooling of the first olein at 6 °C.

(Codex Alimentarius Commission, 2001). Iodine number of the virgin oil was 88.5 while it was 79.8 and 82.1 for the first and second stearin respectively and 86.3 and 92.5 for the first and second oleins, respectively. Iodine number for virgin olive oil is reported to range between 75-94 (Boskou, 2002). It is clear that the olein fractions have higher (though non-significant) iodine numbers than the stearin fractions obtained in the same fractionation step which is due to their lower unsaturated (mono and poly) fatty acid content (Table 2). Sample iodine numbers are in the decreasing order of second olein, virgin oil, first olein, second stearin and first stearin, which is in line with their total unsaturated fatty acid content (Table 2); despite the non-significant differences between them with respect to their unsaturated fatty acid content.

Saponification number was 195.1 for the virgin oil, and 196.1 and 193.5 for first and second stearin respectively compared to 190.0 and 193.1 for the first and second oleins, respectively. Stearin fractions have higher saponification numbers than the olein fractions indicating a lower average chain length of their fatty acids. The results of the fatty acid analysis do not show the short and medium chain fatty acid content (Table 2) as standard fatty acid analyses for olive oil do not include this group of fatty acids. Saponification numbers for olive oil are reported to range between 184-196 (Boskou, 2002). All the samples obtained in this study fall within these values. Solidification point test was not performed on these oil samples as the fractionation process depended on separation of the solid fractions as they solidify at the given temperatures.

These results indicate that the fractionation process did not alter drastically the physical and chemical properties of the oil, and that the fractions obtained still have identity attributes similar to those of the unfractionated virgin olive oil and comply with the Jordanian (JISM, 2009) and other international standards (Codex Alimentatius Commission, 2001) of virgin olive oil.

Differential scanning calorimetry

Results of DSC thermograms are shown in Table 3. All samples exhibited major endothermic peaks between -4.1 and -2.9 °C. These peaks, most likely, correspond to the β' crystals, i.e the major form of triglyceride crystals in oils (Biliaderis, 1983; Kellens *et al*., 2007). Endothermic ΔH values for this peak were highest for the stearin fractions and lowest for the olein fractions. Chiavaro *et al*. (2008) reported a major endothermic peak at -3.5 °C for virgin olive oil. Our results indicate that this peak is at -3.4 °C for the virgin olive oil, while it was shifted to -2.9 and -3.6 °C in case of the first olein and stearin, respectively, and to lower levels of -4.0 and -4.1 °C in case of the second stearin and olein fractions. No endothermic peak was observed at 8 °C as reported by the same authors (Chiavaro *et al*., 2008). However, an exothermic event was observed around -34.6 °C which is probably the same one reported for extra

Table 2. Fatty acid composition of the virgin olive oil and its fractions.[1]

Fatty acid	Number of carbons	Oil sample				
		Mother oil	1st stearin	1st olein	2nd stearin	2nd olein
Myristic	14:0	0.022	0.018	0.026	0.030	0.026
Palmitic	16:0	13.435	14.222	13.792	14.409	12.979
Palmitoleic	16:1	0.680	0.693	0.716	0.748	0.718
Margaric	17:0	0.136	0.139	0.142	0.141	0.151
Heptadecenoic	17:1	0.172	0.158	0.169	0.165	0.174
Stearic	18:0	2.961	3.184	2.868	3.003	2.710
Oleic	18:1	68.919	67.777	68.765	67.999	69.467
Linoleic	18:2	12.130	11.943	11.933	11.955	12.259
Linolenic	18:3	0.715	0.802	0.684	0.701	0.721
Arachidic	20:0	0.434	0.616	0.484	0.449	0.412
Eicosenoic	20:1	0.280	0.330	0.293	0.264	0.270
Lignoceric	24:0	0.114	0.119	0.130	0.135	0.113
Total saturated (%)		17.103	18.297	17.442	18.168	16.391
Total monounsaturated (%)		70.052	68.957	69.942	69.176	70.629
Total polyunsaturated (%)		12.845	12.746	12.616	12.656	12.980
Total unsaturated (%)		82.897	81.703	82.558	81.832	83.609
Total (%)		100	100	100	100	100

[1] Percentage of the total fatty acids.

Table 3. Differential scanning calorimetry data for the major endothermic peaks of whole olive oil and its fractions.

Sample	Enthalpy change (J/g)	Peak temperature (°C)	Onset temperature (°C)	Peak end (°C)	Peak width (°C)	Peak height (mw/mg)
Virgin oil	54.03	-3.4	-7.8	2.2	9.0	1.07
First stearin	63.15	-2.9	-7.8	1.7	9.8	1.39
First olein	52.75	-3.6	-8.0	1.5	8.3	0.98
Second stearin	56.16	-4.0	-9.5	0.8	9.8	1.11
Second olein	51.06	-4.1	-9.9	0.9	8.9	1.15

virgin olive oil by Chiavaro *et al.*(2008) at -27 °C and was attributed to 'super cooling of the oil and the formation of β' crystals'.

The temperature at which the maximum energy absorption was recorded was higher for the stearin than olein fractions which is natural as they have higher melting points. It was also noticed from the thermograms that all samples exhibited minor endothermic peaks around -18 °C which is probably due to the formation of α type of crystals known of their lower melting points (Biliaderis, 1983; Kellens *et al*., 2007). This pattern indicates two types of crystals with different energy absorption levels.

In addition, our results show a minor endothermic peak at about 60 °C in the case of the olein fractions which is likely to be due to the melting of trace amounts of β' crystals. However, the complexity of the triglycerides of olive oil complicates the DSC patterns obtained (Ferrari *et al*., 2007) and hence the interpretation of the different observed events. The complete melting temperature of the crystal is expressed as the peak end temperature shown in Table 3. It is noticed that all fractions melted completely at slightly lower temperatures than the virgin oil and that the fractions of the second fractionation step melted at lower temperatures than those obtained in the first one. In general, with the exception of these minor changes which took place over a narrow range of temperatures, the fractionation process did not result in any major or statistically significant ($P \leq 0.05$) changes in the DSC profiles of the olive oil fractions. Observed differences between our samples and those reported by other researchers can be attributed to varietal and environmental differences (Salvador *et al*., 2003).

DSC profiles and solid fat index

Solid fat index as measured by Dilatometry is reported to correlate strongly with the partial peak area of melting (Menard and Sichina, 2000) obtained from the DSC thermograms. Figure 1 depicts the progress of the melting process during the heating of the oils from -15 to +15 °C as obtained from the DSC thermograms. It is apparent that the two olein fractions melted completely at about 0 °C while the virgin oil and the stearin fractions melted completely at about 4 °C. It is also noticed that there was significantly ($P \leq 0.05$) larger amounts (about twice) of melting of the olein fractions around -5 and 0 °C as compared to that of the virgin oil and the stearin fractions. Stearin fractions were only slightly more solid at 0 °C than the virgin oil. These differences in the solid content of the fractions were observed only within a very narrow range of temperatures which limits the use of the stearins as shortening to those bakery products kneaded at low temperatures like pie crusts and some other types of pastries. However, the olein fraction can be used in salad dressing formulations as substantial amounts of it remained liquid around 0 °C.

Oxidative stability and total phenolic compounds

Rancimat induction times and total phenolic compounds are shown in Table 4. In general, the virgin oil was significantly ($P \leq 0.05$) more stable to oxidation than any of the olein or stearin fractions with an induction period of about 27.5 h compared to 24.48 and 21.19 h for the first stearin and olein fractions, respectively. The second fractionation step resulted in reducing the induction times of the second stearin and olein fractions despite their significantly ($P \leq 0.05$) higher content of the antioxidant-active phenolic

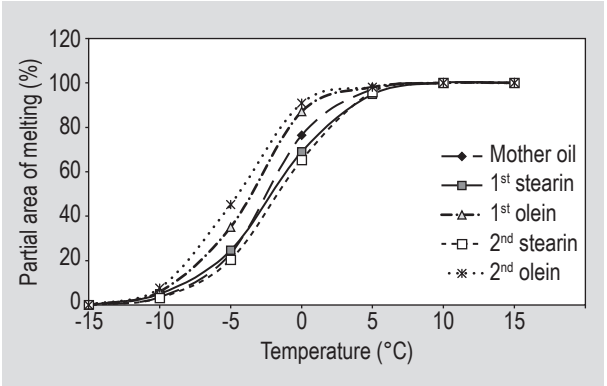

Figure 1. Progress of partial area of melting of the virgin olive oil and its fractions as obtained from the differential scanning calorimetry thermographs.

compounds compared to the virgin oil. However, neither the virgin olive oil content of unsaturated fatty acids nor its content of phenolic compounds explains its superior oxidative stability (Table 4). On the other hand, it is noticed that in both fractionation steps stearin samples exhibited longer induction times than their olein counterparts obtained by the same fractionation steps despite their higher content of phenolic compounds, which is due to the fact that olein has more unsaturated fatty acids than stearin (Table 2).

The total phenolic content of these samples is lower (Table 4) than that reported for virgin olive oil from various Mediterranean countries (60-1000 ug/gm; Tuck and Hayball, 2002). However, regardless of their levels many of the fractions have significantly ($P \leq 0.05$) higher content of phenolic compounds (Table 4) than the virgin oil or the first stearin fraction. This indicates possible redistribution of these compounds which resulted in lowering their levels in the higher melting point fractions (i.e. first stearin and virgin oil).

Fatty acid analysis

Table 2 shows the fatty acid composition of the virgin oil and its fractions. All values are within the limits set up by IOOC and EU (Cercaci *et al*., 2003) as well as Jordanian Standard (JISM, 2009) for virgin olive oil and similar to the values reported for virgin olive oil produced in other countries (Andjelkovic *et al*., 2009; Salvador *et al*., 2003). However, as expected, the stearin fractions contained higher yet not significant ($P \leq 0.05$) levels of saturated fatty acids than the olein fractions. It is also noticed that the second stearin fraction had lower total saturated fatty acids than the first stearin fractions although their levels are within the recommended range set up the IOOC (Cercaci *et al*., 2003). It is also clear that the fractionation step did not affect the fatty acid composition of the fractions obtained in such a way to make them out of conformity with the international standards for the virgin olive oil. Although second olein contains slightly higher levels of unsaturated fatty acids (83.6%) than the other fractions, it remained liquid at the second fractionation temperature.

Table 4. Total phenolic compounds and induction times of the virgin olive oil and its fractions as obtained from the Rancimat[1].

Sample	Total phenolic compounds (ug/gm)		Induction time (h)	
	average	CV (%)	average	CV (%)
Virgin oil	61.0^{cd}	2.30	27.50^{a}	5.41
First stearin	59.5^{d}	1.17	24.48^{b}	5.55
First olein	74.0^{b}	1.93	21.19^{c}	2.07
Second stearin	79.5^{a}	2.66	21.06^{c}	4.27
Second olein	64.5^{c}	1.10	18.77^{d}	4.95

[1] Each value is the average of 4 readings. Averages with the same superscript letters are not significantly different at P=0.05 (LSD test). CV = coefficient of variability.

Sensory evaluation of the virgin olive oil, its fractions, mayonnaise and Italian salad dressing

Analysis of the results of the sensory evaluation revealed no detectable differences between the virgin olive oil and any of its fractions with respect to the sensory attributes set up by IOOC. However, although analysis of the triangle test of the mayonnaise and Italian salad dressing samples prepared from the virgin oil and its fractions revealed no statistically significant ($P \leq 0.05$) differences, the first olein and second stearin samples were preferred by most panellists over those prepared from other fractions or the virgin oil. The colour of the Italian salad dressing was highly acceptable to the panellists and very similar to that of the commercially-prepared dressing, while the mayonnaise colour, although acceptable too, was slightly yellowish compared to the commercially prepared mayonnaise from refined corn oil. However, this was not a problem for the panellists who are used to green-yellowish olive oil in their salads and other dishes. The olive oil flavour was an added advantage to this product by the panellists who value the flavour of olive oil. As the mother oil used was extra virgin and obtained fresh from the press, there was no detectable oxidative flavour by these trained and certified panellists in either of the two products. Both first olein and second stearin fractions had the highest phenol content and showed good stability values of 21 h by the Rancimat test (Table 4). The only problem was the high price of these would-be products compared to those available in the market and prepared from less expensive oils. Furthermore, it was noticed that the salad dressing prepared from the olein samples remained liquid in the refrigerator (6 °C) after one month of storage. Mayonnaise sample prepared from the second stearin fraction did not separate when taken out of the refrigerator after one month of storage while the mayonnaise prepared from the other oil fractions were not as stable and showed various degrees of separation when taken out of the refrigerator.

4. Conclusions

In conclusion, it is possible to obtain new fractions from virgin olive oil without compromising their identity. Some of the new fractions, although have many similar physical, chemical and sensory characteristics, do solidify at lower temperatures which makes them more suitable than the virgin oil for some uses like the production of mayonnaise and Italian salad dressing. By remaining liquid at a lower temperature than the mother oil or the other fractions, the

double fractionated olein can be used in the production of more types of refrigerated salad dressings. A process that opens the door for a number of higher value-added health food products. The low cost and simplicity of the process makes it cost effective which in turn makes the fractions command better prices in the market.

Acknowledgements

The author would like to thank the Deanship of Scientific Research at the University of Jordan for supporting this work. The author also declares no conflict of interest.

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