



## Composition of Fatty Acids and Tocopherols in Cherry and Lychee Seed Oils

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### SUMMARY

The fruit seed from two small fruits namely cherry (*Prunus avium* L.) and lychee (*Litchi chinensis* Sonn.) were analyzed for their oil yields, fatty acids and tocopherols composition. The oil content, extracted with hexane, from cherry and lychee fruit seed was found to be 4.1 and 3.2 %, respectively. Cherry fruit seed oil was characterized by the presence of high content of oleic acid (47.3%), followed by linoleic acid (30.6%) and  $\alpha$ -eleostearic acid (9.2%) while lychee seed oil mainly contained dihydrosterculic acid (46.9%), oleic acid (29.3%) and linolenic acid (3.6%). The ratio of polyunsaturates to monounsaturates to saturates varied considerably between the seed oils tested. Besides, the oils from cherry and lychee exhibited appreciable amount (mg/kg) of  $\alpha$ -,  $\gamma$ - and  $\delta$ - tocopherols with contribution of 126.7, 223.1, 18.2 and 235.8, 97.3, 132.6, respectively. The results of this study advocate the uses of these under-utilized small fruit resources for oil production. The characterized oils, being a good source of unsaturated fatty acids and antioxidant tocopherols, could be explored for edible or oleochemical uses.

**KEY-WORDS:** underutilized oilseeds, fatty acids, minor components, cherry, lychee, tocopherols isomers.



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## INTRODUCTION

There is continuing increase in the demand of vegetable oils due to rapidly growing human population and expansion of oleochemicals industry. In this regard, the world's current vegetable-based oil production has risen to levels as high as 144 million tons per year. To maintaining a steady supply of oils and fats due to an ever-growing market place, it has become important to not only introduce new varieties of oilseeds with higher oil yield but also to search some newer and non-conventional oil sources [1, 2].

Plant seed oils are widely used for food and other industrial non-food applications (such as inks, detergents, paints, coatings, cosmetics, lubricants etc.), depending upon their physicochemical properties which mainly depend upon the fatty acids composition [3, 4]. Saturated fatty acids (palmitic, stearic) have high melting points and are generally linked with negative health impact. On the other hand, unsaturated, especially, the polyunsaturated (linoleic, linolenic) fatty acids, although associated with positive health image, are easily oxidized [3]. From the view-point of technical applications, the fatty acids profile of vegetable oils has been extensively studied [5-7]. As an example, cyclopropanoic fatty acids have been isolated from different plants oils with potential oleochemical applications [8-11]. Tocopherols are one of the minor but valuable nontriglyceric components, which contribute to antioxidant properties of oils and thus enhance their functional food value and shelf-life [6].

Cherries, the members of the *Rosaceae* family, are fairly distinct from their other stone fruit relatives such as plums, apricots, peaches and almonds. Of the two important cherry members: *Prunus avium* L. is the sweet cherry while *Prunus cerasus* L. the sour cherry. Sweet cherry (*Prunus avium* L.) has been cultivated since ancient times, but has attracted recent attention due of its health benefits attributable to the presence of anti-oxidative and pain-relieving bioactive components [12].

A native of south-east Asia, lychee (*Litchi chinensis* Sonn.) is another important fruit now distributed in several parts of the world mainly in subtropical regions. The fruit is accepted by consumers because of its delicious taste and attractive colour. Lychee by-products consist mainly of lychee pericarp and lychee seeds which are discarded as a waste. Some previous studies on biochemical activities of lychee fruit have mainly focused on its pericarp, because it has been found to be a rich source of a multitude of potential antioxidants [13-15]. However, there is no considerable data on lychee seeds, which are commonly used in Chinese Traditional Medicine to relieve neuralgia pain.

World production of lychee is estimated to be around 2.11 million tons, with more than 95% of the world cultivation occurring in Asia. The top five world lychee producing countries are China, India, Taiwan, Thailand, and Vietnam [16]. The total production of cherry was 2033.1 million metric tons in 2007 and major producing countries were Turkey, United States and Iran. In Pakistan: Quetta, Pishin, Ziarat, Kalat, Zhob, Mastung, Loralai and Swat are ideal temperate zones for commercial cherry growing. In Balochistan, cherry is grown on about 897 ha on commercial basis with an annual production of about 1,507 tons [17].

There is growing interest in the search of new fruit seed oils having unique fatty acids and high-value bioactives for specific nutraceutical, cosmeceuticals or functional food uses [2, 6, 7]. According to the estimates millions of pounds of fruit seeds are discarded annually as result of fruit processing and domestic intake [18]. This not only wastes potentially valuable agro-resources but also aggravates an already prevailing serious disposal problem. It is understandable that some of the seeds will be difficult to collect because of the direct consumption of the fresh fruit by consumers, but the bulk of the fruits have been used in food processing plants where the seeds can be easily collected for further processing. To gain the most economical and efficient utilization of such fruit seeds, it is very important to get more information on the properties of the related oils thus produced.

Being the potential fruit seed crops, lychee and cherry, produce a huge quantity of seeds, often discarded as agro-waste. As several of the fruit seed oils are rich source of essential fatty acids and other beneficial bioactives, it is worthwhile to characterize Lychee and cherry fruit seed oils for high-value components. The main objective of the present research was to study the profile of fatty acids and tocopherols components of the seed lipids extracted from fruits of cherry and lychee native to Pakistan.

## MATERIALS AND METHODS

### Materials

Fully ripened fruits of Cherry (*Prunus avium* L.) and Lychee (*Litchi chinensis*) were purchased from local market of Faisalabad, Pakistan during May to July 2013. The seed were removed manually from fruits, washed with tap water and then dried at 40 °C in an oven (EYELA, VOC-300 SD, Tokyo, Japan) for 24 h. All the reagents (analytical and HPLC) used were purchased from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL- $\alpha$ -tocopherol, (+)- $\gamma$ -tocopherol, (+)- $\delta$ -tocopherol], and fatty acid methyl esters (FAMES) were of Sigma Chemical Co. (St Louis, MO, USA).

### Oil extraction

Dried seeds of cherry and lychee fruits were crushed using a domestic blender (TSK-949, WestPoint, France). Fifty grams of the well crushed seeds for each of the fruit species in each batch were extracted in a Soxhlet extractor apparatus. The extraction was carried out with hexane on a water bath for 6 h. After the extraction process completed, the solvent was removed by distilling off under vacuum using a rotary evaporator (EYELA, N-N Series; Rikakikai Co Ltd., Tokyo, Japan).



## GC and GC/MS fatty acid composition

The oils, as fatty acid methyl esters (FAMES), were prepared and analyzed according to IUPAC standard method [19] using a Perkin-Elmer gas chromatograph (model 8700; Norwalk, CT, USA), attached with a flame ionization detector (FID) and split injector (split ratio 1:100). FAMES were separated using RT-2560 capillary column (100 m x 0.25 mm, film thickness 0.20  $\mu\text{m}$ ). The mobile phase consisted of helium that was flushed through the column at a flow rate of 1.5 mL/min. The column initial temperature was maintained at 150  $^{\circ}\text{C}$ , and then linearly programmed to 250  $^{\circ}\text{C}$  at 4  $^{\circ}\text{C}/\text{min}$  (initial and final hold up time were 1 and 5 min, respectively). The unknown FAME compounds were identified based upon the matching of their relative and absolute retention times with those of standard compounds (Sigma-Aldrich Chemical Co. St. Louis, MO). The quantitative data were obtained with the aid of a built-in data-handling program and the amounts of individual fatty acid (FA) were reported as relative percentage of the total peak areas (g/100 g of FAs).

For further authentication, the FAMES were also analyzed using GC-MS. A 6890N Network GC system (Agilent-Technologies; Little Falls, CA, USA), equipped with an inert XL Mass selective detector (Agilent-Technologies 5975), and auto-injector (Agilent-Technologies 7683B series) was employed. The column, the column working/operational conditions and the mobile phase (helium) flow rate were exactly identical to those as were previously selected for GC analysis. An EI (electron ionization) mode with 70 eV ionization energy, mass scanning range 30–550 m/z, injector and MS transfer line temperatures 250 and 260  $^{\circ}\text{C}$ , respectively were employed. The unknown FAMES were identified by comparing their relative retention times with those of authentic standards and further authenticated using their MS spectral profiles compared to those from the NIST mass spectral library of the system.

## Tocopherol content

Tocopherols were analyzed following the AOCS Official Method Ce 8-89 [20] using Finnigan Surveyor HPLC (Thermo Electron Corporation, Waltham, MA) with a Finnigan Surveyor Autosampler Plus and Finnigan Surveyor FL Plus fluorescence detector, set for excitation at 292 nm and emission 394 nm. The column was a normal-phase Microsorb 100 silica (250 x 4.60 mm; 3 $\mu\text{m}$ ; Varian, CA). Of each sample, 10  $\mu\text{L}$  was injected and separated by mobile phase consisted of 7% methyl-tert-butyl-ether in hexane with a flow rate of 0.6 mL/min. The tocopherols were quantified using external calibration for each isomer separately.

## Statistical analysis

Three different samples of each of the cherry and lychee seed were processed and analyzed independently in triplicate. Data are presented as mean values  $\pm$  SD of triplicate determinations. Analysis of variance (ANOVA) was performed using Minitab 2000 Version 13.2 statistical software (Minitab Inc., State College, PA, USA). A probability value ( $p < 0.05$ ) was selected to consider the statistically significant difference of means. **3.**

## RESULTS and DISCUSSION

### Fruits seed oil yield

The cherry seed oil is valued as specialty oil because of its nutritional status [21]. The fruit seeds from cherry and lychee, when extracted using Soxhlet method, yielded 4.1 and 3.2 g/100 g of oil, respectively (Table 1). The seed oil yield in the present study for the cherry fruit was found to be lower than that investigated by Straccia et al. [22] for cherry seed (6.5 g/100 g). When compared with some other non-conventional oil resources, the present fruit seeds oil content (3.2-4.1%) was lower than those of mahaleb cherry (18.5%), blackthorn (16.5%) [23], raspberry seeds (10.7%) [24], grape seeds (6-20%) [25]. Nevertheless, the present fruit seed oil contents are quite close to that investigated for some small fruits seed such as Woods' rose and hawthorn seeds, 3.7 and 3.4%, respectively from Southern Alberta, Canada [2]. According to Hosseinian et al. [26] fat contents in fruits of Manitoba strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry and seabuckthorn ranged from 0.3 to 4.4%, however they did not analyze the lipid contents in the fruit seeds.

### Fatty acid composition of seed oil

The fatty acid composition (g/100 g FA) of cherry and lychee seed oil is summarized in Table 1. Representative GC-MS chromatograms of the said oils are given as Fig. 1 and Fig. 2. The results given in Table 1 showed that two major acids (oleic and linoleic acids) accounted for more than 77% of the total fatty acids in cherry seed oil. Cherry seed oil was rich in unsaturated fatty acids, of which monounsaturated and polyunsaturated fatty acids represented 48.1% and 40.0% of the total fatty acids, respectively. Linoleic, oleic and  $\alpha$ -eleostearic acids accounted for 30.6%, 47.3% and 9.2% of the total fatty acids, respectively. These values of linoleic acid and oleic acid in the examined cherry seed oil from Pakistan were somewhat varied to those investigated by Kamel and Kakuda [18] *i.e.* linoleic acid (35.0%) and oleic acid (52.9%) in Canadian cherry seed oil. The contents of total saturated fatty acids (TSFAs), comprising of palmitic acid (6.4%), stearic acid (3.5%) and arachidic acid (1.1%), accounted for 11.0% of the total fatty acids of cherry seed oil. The concentration of total unsaturated fatty acids (TUFA) (88.1%) in the present analysis of cherry seed oil from Pakistan was comparable to that reported for Canadian cherry seed oils (88.2%) [18] and French cherry seed oil (88.6%) [27].

The level of total essential fatty acids (TEFA), including linoleic and linolenic acids, in the present study, varied slightly from those reported for Canadian cherry seed oils (35%) [18]. Linoleic acid (C18:2), recognized as one of the most important fatty acids in human food because of its protective effects against cardiovascular diseases [28], is present in most of the seed oils. It is well known that dietary fat rich in linoleic acid, apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, also prevents high blood pressure. Linoleic acid derivatives serve as



structural components of plasma membrane and act as precursors of some metabolic regulatory compounds [29]. The presence of one of the essential fatty acids *i.e.* C18:2 in the cherry fruit seed oil makes it nutritionally valuable.

The results of lychee seed oil showed that chiefly it contained cyclic fatty acids namely dihydrosterculic acid and 2-hexyl-cyclopropaneoctanoic acid which accounted for 46.9% and 2.3% of the total fatty acids. The cyclic fatty acids such as dihydrosterculic acid (cyclopropanoid acid; CPA) and sterculic acid (cyclopropenoid acid; CPE), often distributed in bacterial cell membranes, are also present in some seed oils, especially, of the family Bombaceae, Malvaceae, and Tiliaceae [30, 31]. Among the oil seed crops containing the cyclopropene fatty acids (CPE-FAs) and cyclopropane fatty acids (CPA-FAs), lychee (*Litchi sinensis*) seed and cottonseed are the most dominant [30-33]. In close agreement with our present data, it has been investigated that lychee seed oil has about 41% CPA-FAs (dihydrosterculic acid). Some other vegetable seed oils such as Kwai MiPink (46%), Mauritius (45.1%) and Picard (45%) seed oils also had high contents of dihydrosterculic acid [33].

The biological functionalities of cyclic fatty acids (CPA-FAs and CPE-FAs) in plants are still under investigation, however, these might act as antifungal agents [30]. Although CPA-FAs and CPE-FAs may have anti-nutritional effects in animal and poultry diets, the high chemical reactivity of these fatty acids may be potentially useful for different commercial (oleochemical) applications [30]. Lychee seed oil also contained significant amount of oleic acid (29.3%), together with considerable amount of linoleic acid (3.6%) while the saturated fatty acids accounted for 14.6% of total fatty acids. The main saturated fatty acids were palmitic acid (8.8%), and stearic acid (4.1%) along with small contribution of arachidic acid (0.6%).



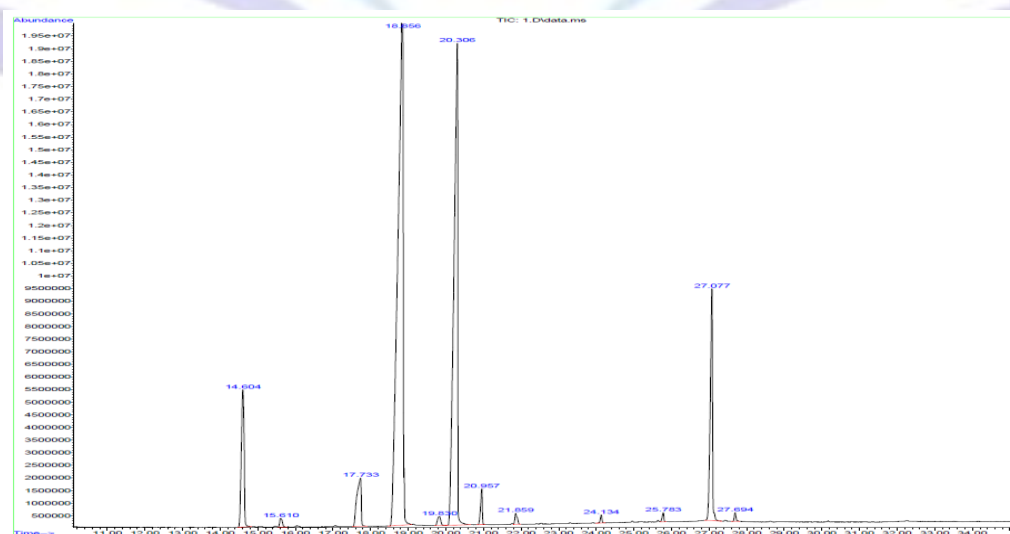
**Table 1** Yield (g/100g) and fatty acid (FA) composition (g/100 g FA) of oils from cherry and lychee seeds

FA	Short Name	Cherry	Lychee
Palmitic acid	C16:0	6.44±0.14 <sup>b</sup>	8.76±0.09 <sup>a</sup>
Cyclopropaneoctanoic acid 2-hexyl	C16:1,9cy	Nd	2.25±0.05 <sup>a</sup>
Palmitoleic acid	C16:1	0.38±0.02 <sup>a</sup>	Nd
Stearic acid	C18:0	3.49±0.10 <sup>a</sup>	4.07±0.04 <sup>a</sup>
Oleic acid	C18:1,9 c	47.28±1.26 <sup>a</sup>	29.32±0.54 <sup>b</sup>
Vaccenic acid	C18:1n7	Nd	0.95±0.01 <sup>a</sup>
Dihydrosterculic acid	C19:1cy	Nd	46.88±0.82 <sup>a</sup>
Linoleic acid	C18:2	30.57±0.64 <sup>a</sup>	0.23±0.01 <sup>b</sup>
Linolenic acid	C18:3 9c,12c,15c	0.23±0.06 <sup>b</sup>	3.59±0.02 <sup>a</sup>
α-Eleostearic acid	C18:3 9c,11t,13t	9.20±0.17 <sup>a</sup>	Nd
Arachidic acid	C20:0	1.06±0.03 <sup>a</sup>	0.58±0.01 <sup>b</sup>
11-Eicosenoic acid	C20:1 11c	0.42±0.04 <sup>a</sup>	Nd
Behenic acid	C22:0	0.19±0.01 <sup>b</sup>	0.35±0.04 <sup>a</sup>
Lignoceric acid	C24:0	Nd	0.80±0.10 <sup>a</sup>
Others	————	0.74±0.03 <sup>b</sup>	2.22± 0.08 <sup>a</sup>
SFA	————	11.18 <sup>b</sup>	14.56 <sup>a</sup>
MUFA	————	48.08 <sup>a</sup>	30.27 <sup>b</sup>
PUFA	————	40.0 <sup>b</sup>	52.95 <sup>a</sup>
Oil content (g/100g of fruit seed)		4.1±0.20 <sup>a</sup>	3.2±0.15 <sup>b</sup>

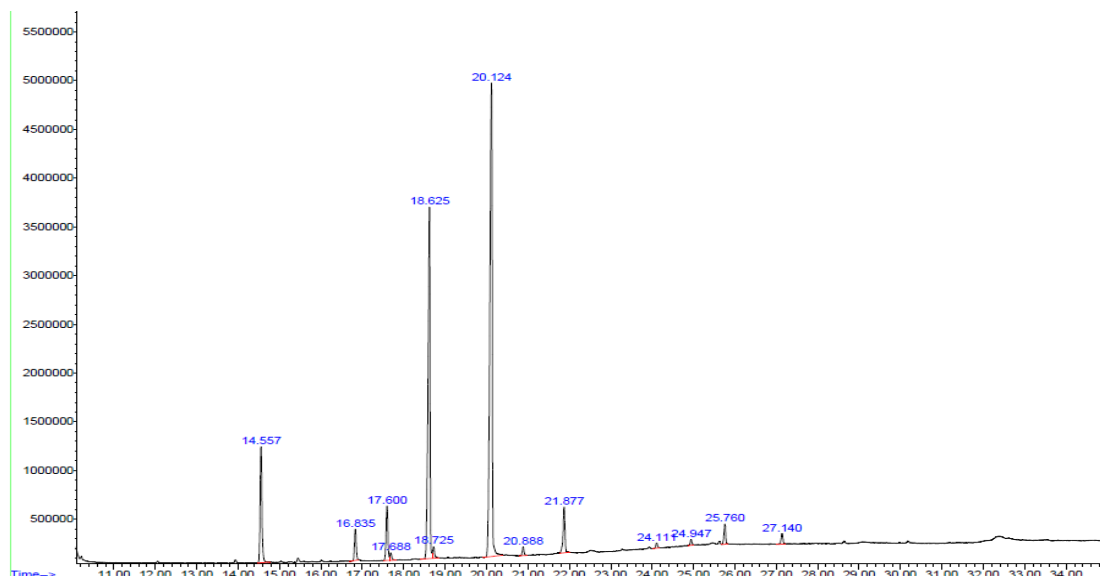
ND: not detected; C:cis: cy: cyclic

Values are means ± SD of three samples of each fruit, analyzed individually in triplicate

Mean in same Row followed by different superscript letters indicate significant differences (p<0.05) between the type of fruits



**Fig. 1.** Gas chromatography/mass spectrometry chromatogram of typical cherry (*Prunus avium* L.) seed oil



**Fig. 2.** Gas chromatography/mass spectrometry chromatogram of typical lychee (*Litchi chinensis*) seed oil

### Tocopherol composition

The data for the quantification of tocopherols ( $\alpha$ ,  $\gamma$ , and  $\delta$ ) in the tested fruit seed oils are presented in Table 2. The total tocopherols in cherry seed oil are 368 mg/kg while in lychee is 466 mg/kg. The contents of total tocopherols in these two presently investigated under-utilized fruit seed oils is lower than some other non-conventional seed oil such as *Cucurbita pepo* (454-709 mg/kg) [34], Malaysian *Cucurbits*, bitter melon, Kalahari melon and pumpkin seed oils (806.5-1350.2 mg/kg) [35]. Similarly, the contents of total tocopherols reported by Anwar et al. [2] in selected similar small fruits namely chokecherry, thorny buffaloberry, Woods' rose and hawthorn seed lipids with contribution 595, 897, 2,358 and 2,837 mg/kg, respectively, were notably higher than our present analysis. On individual tocopherol isomers basis, the content of  $\alpha$ -tocopherol in the seed oil of cherry was 127 while in lychee 236 mg/kg. These levels are lower than those determined in most of the conventional edible oils, such as cottonseed (338 mg/kg) and maize (282 mg/kg) [36]. According to a recent study by Yılmaz & Gökmen [37], Turkish sour cherry seed oil contains  $\alpha$ -tocopherol between 66 - 95 mg/kg, depending on the extraction method, which is quite lower than that determined in the Pakistani cherry seed oil in the current study. The study conducted by Ivanov and Aitzetmueller [38] shows that seed oil of *Litchi chinensis* contained  $\alpha$ -tocopherol (9361 mg/kg) much higher than the present study. The concentration of  $\gamma$ -tocopherol (223 mg/kg) in the investigated cherry seed oil was within the range reported for sour cherry seed oil which is between 183 – 274 mg/kg depending on the extraction protocol [37]. Whereas  $\gamma$ -tocopherol content in the lychee seed oil is quite low (97 mg/kg), but comparable to the value reported for the Vietnamese variety (105 mg/kg) [39]. The concentration of  $\delta$ -tocopherol in cherry and lychee seed oil was 18 and 133 mg/kg, respectively.

High amount of vegetable oil tocopherols could be of interest for the isolation and purification of naturally occurring tocopherols and tocotrienols for application in dietary, pharmaceutical, or biomedical products [38]. Currently, there is an increasing demand for edible oils with high contents of medicinally valuable fatty acids and minor antioxidant components for functional food applications. In this context, studies have been conducted to evaluate the chemical composition and potential nutraceutical applications of fruit, spice, and herbal seed oils [40].

**Table 2** Tocopherol contents (mg/kg) of cherry and lychee seed oils

Tocopherol	Cherry	Lychee
$\alpha$ -Tocopherol	126.7 $\pm$ 5.1 <sup>b</sup>	235.8 $\pm$ 10.6 <sup>a</sup>
$\gamma$ -Tocopherol	223.1 $\pm$ 14.2 <sup>a</sup>	97.3 $\pm$ 6.4 <sup>b</sup>
$\delta$ -Tocopherol	18.2 $\pm$ 1.0 <sup>b</sup>	132.6 $\pm$ 4.9 <sup>a</sup>
Total tocopherols	368.0 <sup>b</sup> $\pm$ 16.4	465.7 <sup>a</sup> $\pm$ 15.0



Values are mean  $\pm$  SD of three seed oils of each cherry and lychee, analyzed individually in triplicate

Means in the same row followed by different superscript letters indicate significant differences ( $p < 0.05$ ) between the types of fruits

## CONCLUSIONS

The seed oil of cherry was rich in oleic and linoleic acid; both of these fatty acids are valued in human nutrition due to the potential health benefits, especially against heart diseases. Lychee seed oil was rich in cyclic fatty acids and thus can be explored as raw material for oleochemical applications. Both of the fruit seed oils tested also contained considerable amount of antioxidant tocopherols contributing towards better oxidative stability and nutritive status of these oils. The findings of this study could help explore the uses of under-utilized lychee and cherry fruit seeds for oil extraction and value-addition. From future perspectives, research is needed to further elucidate and characterize the structure and chemical composition of the additional minor bioactives in these seed oils to develop novel formulations for optimum human nutrition.

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