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Research Article

INFLUENCE OF PROCESSING ON PHYSIOCHEMICAL, NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION OF *CARISSA SPINARUM* (KARONDA) FRUIT

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ABSTRACT

Objective: In this study, the effects of three different processing methods, sun drying, freezing, microwave drying, and fresh fruits of *Carissa spinarum* in terms of physical, nutritional and phytochemical composition have been studied.

Methods: These local variety of fruits were selected from Himachal (India). These fruits were obtained fresh, cleaned, and washed prior to selected techniques.

Results: As we see from, the results in Tables showed that drying techniques improved the protein, carbohydrates, ash content, dietary fiber (neutral detergent fiber, acid detergent fiber, cellulose, hemicelluloses, and lignin), total phenol, antioxidant activities, and mineral content. Drying destroyed total flavonoids, tannin, alkaloid, and anthocyanin content.

Conclusions: The findings of the research is clearly indicated that some processing methods are proved good for more nutrient retension as compared to others. This study aim to make consumers aware about the effects of processing methods on nutritional value of fruits and study will help people to generate awareness for the intake of these underutilized fruits in their daily diet increase nutritional status in a better way.

Keywords: Sun drying, Freezing, Microwave drying, *Carissa spinarum.*

INTRODUCTION

Many studies have demonstrated that daily intake of fruits is associated with the diminution of chronic degenerative diseases [1]. However, in another investigations, it has been also observed that fruits has medicinal properties that may reduce the risk of many disorders such as constipation, diabetes, heart diseases, and obesity [2,3]. Indian diets mostly rich in major food groups such as cereals, pulses, green leafy vegetables, roots, tubers, other vegetables, fruits, oil seeds, spices, and condiments. Fruits are consumed in various forms such as fresh, dried, frozen, or canned [4]. Many types of fruits might be grown in India particularly, underutilized fruits, such as aonla, tamarind, karonda, fig, citron, and jackfruit, are the main sources of livelihood for the poor and contribute to overcome the problem of malnutrition [5]. *Carissa spinarum* is a species of flowering shrub and starts in the month of January-February and fruits mature in May-June. It is cultivated in Siwalik Hills, India mainly in Rajasthan, Gujarat, Bihar, Uttar Pradesh, Himachal Pradesh and distributed in Sri Lanka, Indonesia, Malaysia, Myanmar, and Pakistan [6]. It is rich in vitamins, mineral, calcium, fats, and dietary fiber [7]. These fruits are cheaper, fresher but short shelf life. Therefore, processing of fruits need to be done to increase their shelf life. The purpose of the research was to investigate the effect of processing methods such as sun drying, freezing and microwave drying on physical, nutritional, and phytochemical composition of fresh selected whole *C. spinarum* fruit (local variety) of Himachal Pradesh.

METHODS

Sample selection

Ripened fresh fruits (15 g individual packages for different selected processing methods) were collected from orchard of a local cultivar of *C. spinarum* in Bilaspur (Himachal Pradesh), from January 2014 to June 2015.

Sample preparation

Sorting

Fresh, black and non-inspected fruits were collected and discolored, decayed fruits were discarded before washing.

Washing

The whole selected fruits were washed 3 times with distilled water to remove unwanted dirt particles and air dried to remove extra water and after drying fruits were then weighed and divided equally into four batches like fresh, sun drying, freezing, and microwave drying.

Drying techniques

The selected fresh whole fruits were subjected to three different methods.

Sun drying

C. spinarum (15 g) fruits were distributed on the stainless steel trays and dried under direct sunlight at temperature between 25°C and 30°C, for 5 days with about 36 hrs of daylight, between 15 July and 20 July, 2015.

Freezing

In freezing, the selected whole fresh (15 g) *C. spinarum* fruits were put in the lyophilized condition at –20°C freezer until weight become constant.

Microwave drying

Fresh *C. spinarum* (15 g) fruits were placed in a single layer in a Pyrex petri dish and heated in an microwave oven (Sharp R-248e; 800 W) for 3 minutes and 15 seconds until weight become constant. After heating, the fruit was allowed to cool at room temperature. After cooling a second time, the weight was measured to check the percent-age of weight loss.

Sample extraction

Solvent extraction was done with methanol. 1 g dried powder (for three selected treatments) and 1 g fresh fruits of *C. spinarum* were weighed, separately mixed with 80% methanol (v/v) at a ratio 1:4 in a conical flask (wrapped with aluminum foil) and agitated at 200 rpm, at 50°C with the aid of an orbital shaker (Heidolph Unimax 1010, Schwabach, Germany) for 2 h. Mixture was filtered through a whatman filter paper No.4 and a clear solution obtained for further analysis [8].

Physical properties

Length and width was measured by vernier calliper method by Mohsenin [9]. Moreover, density was measured by toluene displacement method [10,11].

Proximate analysis

The selected whole fruits fresh and dried under the influence of selected methods were analyzed for proximate composition (moisture, ash, fat, protein, carbohydrates, dietary fiber). Proximate analysis of whole fruits were analyzed in triplicates. Moisture, ash and fat content and protein was determined by micro-kjedahl [12]. Carbohydrates was determined by Anthrone method [13]. Dietary fiber (cellulose, hemicelluloses and lignin) were determined by Van Soest method [14]. Mineral content were estimated by gas chromatography-mass spectrometry [15].

Phytochemical composition

The phenolic content in the fruit was estimated by Folin–Ciocalteu method given by Thimmaiah [16]. Total antioxidant activity measured by Kekuda *et al*. [17]. Flavonoid estimated by spectrophotometer by Luximon-Ramma [18]. Anthocyanin was determined by pH-differential method [19]. Alkaloid was estimated by Herborne [20]. Tannins was determined using Spectrophotometric methods [21].

Statistical analysis

Statistical analysis were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 16.0 for windows. Data are represented as mean and standard deviation. All determinations were done at least in triplicate and average were calculated. Where appropriate data were subjected to statistical Analysis of Variance to determine the significance of treatment relationship. The confidence limit used in this study were based on (95%) probability ($p<0.05$).

RESULTS AND DISCUSSION

The data contained physical properties, proximate and phytochemical composition of *C. spinarum* shows in Tables 1-6 and Figs. 1-4.

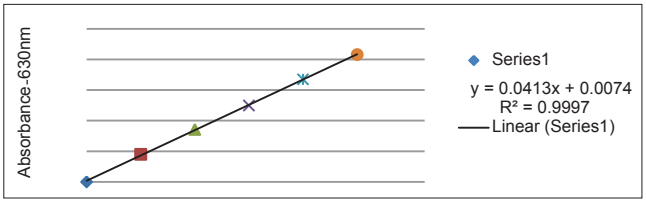

Fig. 1: Standard curve for carbohydrates

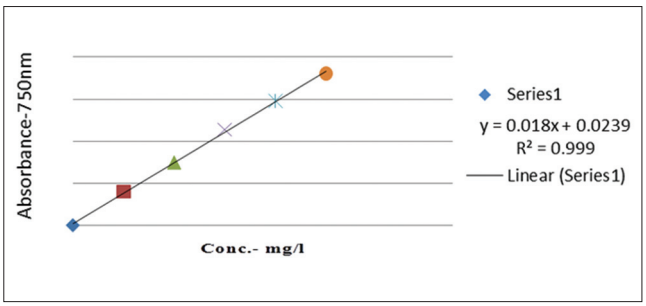

Fig. 2: Standard curve for total phenol

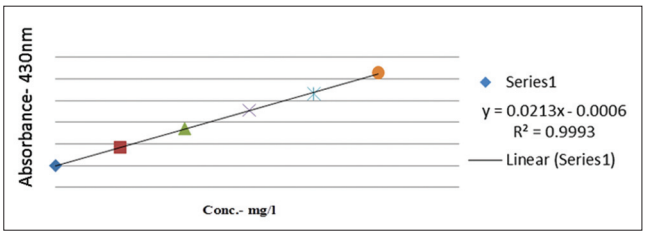

Fig. 3: Standard curve for total flavonoids

Table 1: Physical properties of *C. spinarum*

Drying methods	Fresh	Sundried	Freezing	Microwave
Length (mm)	7.46±0.05^{a}	6.06±0.05^{b}	6.36±0.05^{cd}	5.96±0.05^{dbe}
Width (mm)	4.54±0.00^{a}	3.76±0.05^{b}	3.86±0.05^{cb}	3.26±0.05^{dce}
Density (g/cc)	0.61±0.01^{a}	0.62±0.00^{a}	0.60±0.00^{a}	0.64±0.01^{bac}

Values are expressed as mean±SD (n=3) of triplicate measurement, different letters in the column indicated significant differences at $p<0.05$, SD: Standard deviation, *C. spinarum: Carissa spinarum*

Table 2: Nutritional composition of *C. spinarum*

Drying method	Fresh	Sun dried	Freezing	Microwave
Moisture (%)	81.05±1.97^{a}	16.86±0.75^{b}	82.06±2.19^{ad}	16.83±0.40^{cbe}
Ash (%)	2.46±0.06^{a}	2.51±0.05^{a}	2.48±0.07^{a}	2.50±0.06^{a}
Carbohydrates (%)	18.66±0.25^{a}	60.51±0.00^{b}	18.16±0.59^{a}	61.81±0.01^{cde}
Fat (%)	1.30±0.01^{a}	1.50±0.03^{b}	1.29±0.02^{ac}	1.51±0.01^{cbd}
Protein (%)	2.07±2.04^{a}	2.41±0.33^{a}	2.04±0.04^{a}	2.51±0.33^{a}

Values are expressed as mean±SD (n=3) of triplicate measurement, different letters in the column indicated significant differences at $p<0.05$, SD: Standard deviation, *C. spinarum: Carissa spinarum*

Table 3: Dietary composition of *C. spinarum*

Drying method	Fresh	Sun dried	Freezing	Microwave
NDF (%)	25.43±0.05^{a}	25.56±0.66^{a}	25.26±0.05^{a}	26.23±0.05^{b}
ADF (%)	16.03±0.41^{a}	16.13±0.66^{a}	15.96±0.05^{a}	16.50±0.05^{b}
Hemicellulose (%)	9.40±0.51^{a}	9.43±0.98^{a}	9.20±0.10^{a}	9.66±0.15^{a}
Cellulose (%)	14.05±0.13^{a}	14.67±0.54^{a}	12.97±0.00^{b}	14.89±0.09^{abc}
Lignin (%)	3.10±0.05^{a}	3.20±0.05^{b}	3.00±0.10^{ac}	3.33±0.05^{cbd}

Values are expressed as mean±SD (n=3) of triplicate measurement, different letters in the column indicated significant differences at $p<0.05$, SD: Standard deviation, *C. spinarum: Carissa spinarum*

Table 4: Phytochemical composition of *C. spinarum*

Drying method	Fresh	Sun dried	Freezing	Microwave
TP (mg TAE/g)	5.31±0.21^{a}	5.50±0.00^{a}	5.11±0.01^{ab}	5.74±0.00^{bac}
TF (mg QE/100 g)	0.44±0.00^{a}	0.31±0.00^{b}	0.52±0.00^{cd}	0.32±0.00^{dbe}
DPPH (%)	34.45±1.725^{a}	34.47±1.725^{a}	30.83±0.889^{a}	34.48±0.998^{a}
FRAP (%)	58.63±0.06^{a}	58.68±0.16^{a}	55.36±5.85^{a}	58.79±10.30^{a}

Total phenolic content is expressed as mg of tannic acid equivalents in 100 g of dried sample (mg TAE per 100 g dried sample), total flavonoid content is expressed as mg of quercetin equivalents in 100 g of dried sample (mg QE per 100 g dried sample), TP, TF and antioxidant activity (DPPH and FRAP), TP: Total phenolic content, TF: Total flavonoids content, FRAP: Ferric reducing scavenging activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl, *C. spinarum: Carissa spinarum*, SD: Standard deviation

Table 5: Anti-nutritional content and anthocyanin content of *C. spinarum*

Drying method	Fresh	Sun dried	Freezing	Microwave
Tannin g/100 g	0.98±0.01^{a}	0.96±0.01^{a}	0.95±0.00^{a}	0.97±0.02^{a}
Alkaloid/100 g	1.94±0.00^{a}	1.92±0.01^{b}	1.90±0.00^{cb}	1.92±0.00^{dbc}
Anthocyanin g/100 g	54.03±0.00^{a}	53.43±0.00^{a}	55.20±2.48^{a}	53.39±5.02^{a}

Total anthocyanin content is expressed as mg of cyanidin-3-glucoside equivalents in 1 g of dried sample (mg C-3-GE g^{-1} dried sample), *C. spinarum: Carissa spinarum*, SD: Standard deviation

Table 6: Mineral content of *C. spinarum*

Drying method	Fresh	Sun dried	Freezing	Microwave
Calcium-(mg/100 g)	29±0.57^{a}	275.674±0.00^{b}	28.9±0.00^{ac}	286.358±0.00^{cde}
Iron-(mg/100 g)	3.45±0.00^{a}	12.43±0.00^{b}	3.4±0.05^{cd}	12.82±0.00^{def}
Phosphorus-(mg/100 g)	32.1±0.05^{a}	106.205±0.00^{b}	31.9±0.05^{cd}	108.504±0.00^{dce}

Values are expressed as mean±SD (n=3) of triplicate measurement, different letters in the column indicated significant differences at p<0.05, SD: Standard deviation, *C. spinarum: Carissa spinarum*

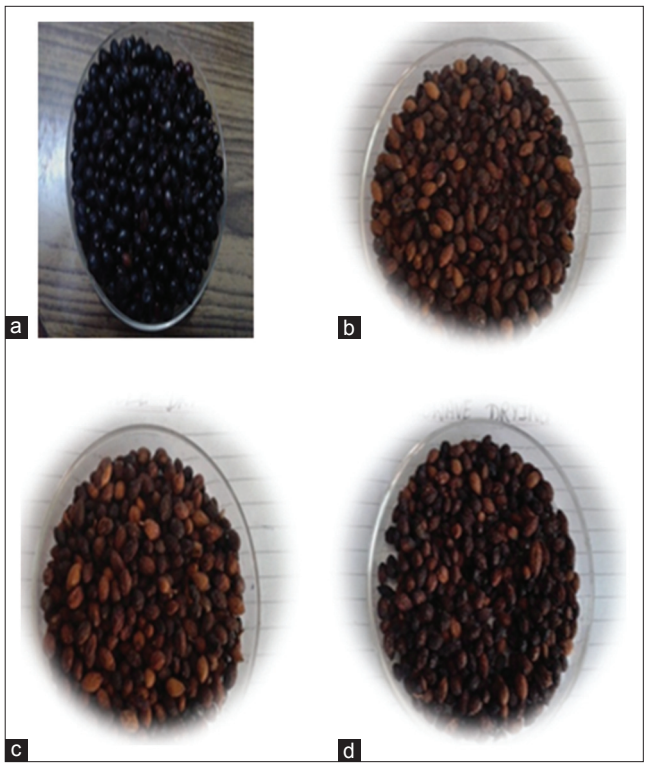

Fig. 4: Fruit sample picture under the treatment of selected methods, (a) Fresh, (b) Sundried, (c) Freezed, (d) Microwave dried

The physical properties *viz.* length, width, and density were determined and results are depicted in (Table 1). Length was reported as 7.46 mm, 6.06 mm, 6.36 mm, and 5.96 mm for fresh, sun dried, freezing, and microwave dried. Moreover, width ranged from 4.54 mm, 3.76 mm, 3.86 mm, and 3.26 mm for fresh, sun dried, freezing, and microwave dried, respectively. Similar results were given by Amreen [22]. Length and width decreased after drying with decreasing the sphericity of the fruits [23]. Length and width decreased in microwave as compared to sun drying because of decreased more moisture content by high heating intensity in microwave. Similar results with our study, i.e., length and width decreased as the moisture content decreased [24]. Freezing also showed significant reduction in the length and width due to storage period [25]. In case of density, it was mentioned as 0.61 g/cc, 0.62 g/cc, 0.60 g/cc and 0.64 g/cc for fresh, sun dried, freezing, and microwave dried. Khuzma, reported similar results [26]. Result revealed that density of fresh fruit was less as compared to dried because of increased density during drying process due to the variation in the mass, volume and structure of the cell wall and with the removal of water [27,28]. Similar findings were given by Pacco *et al.* [29]. Density increased in microwave drying as compared to sun drying due to the constant increasing drying rate with increasing microwave output power density of fruit. In microwave drying density of fruit increased with increased 35 per cent drying rate [30]. In the present study, the density decreased after freezing. Frozen state the density of fruits decreased approximately 5.2-6.8% as compared to unfrozen state [31]. The results of moisture, ash, carbohydrates, fat and protein that were produced using different processing methods are depicted in Table 2. The moisture content was determined and reported as 81.05%, 16.86%, 82.06% and 16.83% for fresh, sun dried, freezing and microwave dried. These results were consistent with the findings of [32,33]. Microwave dried fruit contained less moisture content as compared to sun dried because of microwave energy is rapidly absorbed by water molecules and resulted, rapid evaporation of water that caused higher drying rates [34]. Freezed sample contained more moisture content as compared to fresh due to syneresis [35]. Ash content was mentioned as 2.46%, 2.51%, 2.48% and 2.50% for fresh, sun dried, freezing and microwave dried. Mishra and Gupta, given similar results [36]. After drying ash content increased due to removal of moisture content [32]. Carbohydrates content reported as 18.66%, 60.51%, 18.16% and 61.81% for fresh, sun dried, freezing and microwave dried. Ara given similar results [37]. Fat content was given as 1.30%, 1.50%, 1.29% and 1.51% for fresh, sun dried, freezing and microwave dried. Morton showed consistent results [38]. Protein

reported as 2.07%, 2.27%, 2.51% and 2.04% for fresh, sun dried, freezing, and microwave dried. Similar results have been obtained by Mahapatra *et al.* [35]. After drying the ash, moisture, carbohydrates and fat content in fruits increased 4.5 times [39] due to removal of moisture content, which is directly related to increase the concentration of nutrients [38]. Microwave dried sample showed better preservation of the nutrients as compared to sun dried sample because sun drying caused reduction in the nutritional contents due to prolonged heating [40,41]. In case of fruits, freezing decreased the ash content from 0.98 g/100 g to 0.34 g/100 g, fat content from 0.37 g/100 g to 0.33 g/100 g and protein content from 0.69 g/100 g to 0.41 g/100 g. After freezing ash, fat and protein content was decreased due to the loss of some nutrients by the presence of some enzymes in fresh fruits [42-45]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and lignin, expressed on wet and dry weight basis, are depicted in (Table 3). The NDF reported as 25.43%, 25.56%, 25.26% and 26.23% for fresh, sun dried, freezing and microwave dried. ADF given as 16.03%, 16.13%, 15.96% and 16.50% for fresh, sun dried, freezing and microwave dried. Hemicellulose content reported as 9.40%, 9.43%, 9.20% and 9.66% for fresh, sun dried, freezing and microwave dried. Cellulose content reported as 14.05%, 14.67%, 12.97% and 14.89% for fresh, sun dried, freezing and microwave dried. Lignin content mentioned as 3.10%, 3.20%, 3.00% and 3.33% for fresh, sun dried, freezing and microwave dried. Rani and Kawatra reported consistent findings with our results [46]. Dietary fiber production take place due to the composition of cell wall by different drying methods [47]. After drying, the dietary fiber increased either may be due to reduction of the moisture content or by enzymatic break down of substances into soluble compounds [48]. After freezing, NDF, ADF, hemicellulose, cellulose and lignin content reduced due to degradation of cell wall components, i.e., cellulose, hemicellulose, pectin and lignin. Furthermore, degradation of the polysaccharides tissues also caused apparent reduction in the fiber [49]. The total phenol content was reported as 5.31 mg TAE/100 g, 5.50 mg TAE/100 g, 5.11 mg TAE/100 g and 5.74 mg TAE/100 g for fresh, sun dried, freezing and microwave dried are depicted in Table 4. Pewlong given similar result [50]. Total phenolic content either may be increased or decreased after drying, depending not only on the cultivar, but also on the production system used, conventional or organic [51]. However, in the presenting study, phenolic content increased after drying due to loss of moisture [52] and Slatnar reported similar findings [53]. Drying responsible to release the bond phenolic compounds from matrix during the breakdown of cellular constitutents [54]. Drying at low temperature resulted reduction in the phenolic content [55] and long drying time might have destroy some phenolic compounds [56]. Microwave drying increased the phenolic content as compared to sun drying due to less heating duration in the microwave might have required to increased the phenolic content [57]. However, sun drying required large drying period for which fruit sample is exposed to the atmospheric oxygen that caused the reduction in ascorbic acid and phenolic compounds, etc. [58]. Freezing decreased the phenolic content due to either oxidation or leaching of water soluble phenolic compounds [59]. The total flavonoids content mentioned as 1.53 mg QE/100 g, 0.31 mg QE/100 g, 0.53 mg QE/100 g and 0.32 mg QE/100 g for fresh, sun dried, freezing and microwave dried. Similar findings were reported by Itankar [60]. Fresh sample contained more flavonoid as compared to dried because of thermal degradation of flavonoids during processing [61,62]. Heating may breakdown some phytochemicals which affect cell wall integrity and caused a migration of some flavonoids component [63]. Thermal degradation occurred during processing in the presence of oxygen by direct oxidation mechanism or through the action of oxidizing enzymes i.e. polyphenol oxidase polyphenoleoxidase. Degradation of flavonoid is occurred not only due to temperature and heating, it may also depend on other parameters such as pH, the presence of oxygen, and the presence of other phytochemicals in the medium [64]. Less degradation of flavonoid occurred in microwave drying as compared to sun drying due to increased microwave output power [65]. The flavonoid content of fruits decreased at lower temperature during heating. Flavonoid content (flavonols) increased after freezing due to the presence of 35% more quercetin derivatives in frozen fruits as compared to fresh, which led to increase the extractability and hydrolysis of quercetin [66]. Antioxidant content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was reported as 34.45%, 34.47%, 30.83%, and 34.48% for fresh, sun dried, freezing, and microwave dried. Prakash reported similar result [67]. Moreover, Ferric reducing scavenging activity (FRAP) was mentioned as 58.63%, 58.69%, 55.36% and 58.79% for fresh, sun dried, freezing, and microwave dried. Salar and Dhall, reported similar results [68]. FRAP used to determine the capacity of the plant extract to donate electron to $Fe3^{+}$ and reduce it to $Fe2^{+}$ ion. Higher FRAP value, means higher the antioxidant activity [69]. Radical scavenging activity enhanced after thermal treatment due to the inhibition of oxidative enzymes and destruction of the cell wall which release the antioxidant compounds [70]. Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but mainly due to their stable radical intermediates, which prevent the oxidation [62,71]. Microwave dried sample contained higher antioxidant activity due to the release of a free phenolic fraction. Microwave processing increased the antioxidant activity [72] because of enhancement of antioxidant properties of naturally occurring compounds such as Millard reaction product that have antioxidant activity [73]. Study showed that the release of phenolic compounds after microwave drying resulted to enhance the antioxidant activity in fruit extracts [74]. Microwave dried sample contained more antioxidant activity as compared to sun dried sample because of increased microwave output power [75]. In result, freezing fruits have lesser antioxidant due to cell wall disruption, which released the oxidative and hydrolytic enzymes that can destroy antioxidants in fruits [76]. Frozen fruits have lower level of antioxidant activity (60-80%) as compared to the fresh [77]. Table 5 depicted that total tannin content was 0.98 g/100 g, 0.96 g/100 g, 0.95 g/100g and 0.97 g/100 g for fresh, sun dried, freezing and microwave dried. Gupta given similar findings. Drying process decreased the tannin content due to thermal degradation and extraction which led to release the tannin content from the cell matrix by the breakdown of bonds with proteins [78]. Heating caused the loss of tannin content due to oxidation of bioactive compounds. Soluble tannin is responsible for the astringency sensation, during freezing soluble tannin coagulates and become insoluble [31,79]. Freezing also decreased the tannin content due to the change of chemical properties of tannin and dehydration of colloidal substances [80]. The alkaloid content was reported as 1.94 g/100 g, 1.92 g/100 g, 1.90 g/100 g and 1.92 g/100 g for fresh, sun dried, freezing and microwave dried. Similar reports were given by Gupta [48]. Drying process decreased the alkaloid content as compared to fresh because of thermal breakdown affect the integrity of cell structure and resulted migration of components, leading losses by various chemical reactions (involving enzymes, light and heat). In microwave drying less degradation of alkaloid and tannin content as compared to sun drying due to the absence of sunlight and low oxygen [81]. Freezing decreased the alkaloid content as compared to the thermal treatment and similar results were reported by Atlabachew [82]. Anthocyanin mentioned as 54.03 mg/100 g, 53.43 mg/100 g, 55.20 mg/100 g, 53.39 mg/100 g for fresh, sun dried, freezing and microwave dried. Similar findings were given by Pewlong [83]. Drying process decreased the anthocyanin content [84] due to various factors such as temperature, presence of oxygen, metal ion, co-pigmentation, pH and light [85]. Microwave drying contained lesser anthocyanin as compared to sun drying. Due to fast heating process in the microwave led to thermal degradation of the anthocyanin by the production of heat from within cells as well as from the outside by radiation, conduction and convection as compared to sun drying because UV irradiation is the non-thermal factor for the color stability of anthocyanins content [36,86]. Freezing increased the anthocyanin extraction due to the cellular disruption in fruits. Moreover, enhanced the release of membrane bound anthocyanin as compared to heating, freezing slightly increased the anthocyanin content and induced the formation of ice crystals that favors localized concentration of solutes (phytochemicals) reallocation of water molecules in the cell structure.

During freezing large amount of ice crystals formed and caused lesser degree of cell disruption [87].

Table 6 reported that the calcium content was as 29.0 mg/100 g, 297.357 mg/100 g, 28.9 mg/100 g and 286.358 mg/100 g for fresh, sun dried, freezing and microwave dried. Similar consistent findings were given by Ara [88]. Iron content reported as 3.45 mg/100 g, 12.43 mg/100 g, 3.4 mg/100 g and 12.82 mg/100 g for fresh, sun dried, freezing and microwave dried. Similar findings were given Dalal [89]. Phosphorus content was ranged from 32.1 mg/100 g, 106.205 mg/100 g, 31.9 mg/100 g and 108.504 mg/100 g for fresh, sun dried, freezing and microwave dried. "CSIR New Delhi" gave similar results with our study [90]. Dried fruits contained more mineral calcium, phosphorus and iron compared to fresh because of increasing dry matter content. Microwave drying fruits contained higher mineral content as compared to sun drying. This might be due to the conversion of energy into heat in the microwave drying process which results in higher temperature. In case of freezing, mineral content decreased 10% and 45% due to leaching of the mineral content. It may be due to during freezing residual soil particles (mineral rich soil) washed off which are left on the surface of fruits [56].

CONCLUSION

This study conclude that these selected methods: Sun drying, microwave drying and freezing have a significant impact on the physicochemical, nutritional and phytochemical properties. Compare to fresh and freezing, drying method would be used to produce good quality dried fruit in terms of protein, carbohydrates, ash content, dietary fiber (ADF, NDF, cellulose, hemicelluloses, pectin), anti-nutritional content (tannin, alkaloid, and minerals. Purpose of the study is to generate awareness among people about the influence of processing methods on fruits and to increase the intake of underutilized fruits in their daily diet.

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