Journal of Pharmaceutical Research International

33(60B): 3986-3993, 2021; Article no.JPRI.82554
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Nutrient Composition and Radical Scavenging Activities of Watermelon Seed-based Nutribar

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i60B35103

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/82554

Original Research Article

Received 02 December 2021
Accepted 27 December 2021
Published 28 December 2021

ABSTRACT

Aims: The present study is aimed to develop watermelon seed nutribars (WMSNB) and to determine proximate content, antioxidant activity, free fatty composition and also sensory evaluation in order to create awareness of its potential nutritional values and increase its consumption in the form of nutraceuticals, functional foods and pharmaceuticals.
Study Design: Proximate analysis, total phenolic, antioxidant activity and free fatty acid profile was carried by using well established *in-vitro* assay.
Place and Duration of the Study: Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research. Kolar, between June 2021 and November 2021
Methodology: The developed nutribars samples were evaluated for proximate analysis (moisture, crude protein, ash, total fat, crude fibre, and total carbohydrate), total phenolic, antioxidant activity by using DPPH assay, free fatty composition by using GC-MS, and sensory characteristics based on hedonic scale point. The ingredients used for preparation of WMSNB were Watermelon seeds (100gm), jaggery (50gm), coconut flakes (15gm), sesame seeds (10gm) and ghee (5gm). Peanut nutribars (PNB) were also developed by replacing Watermelon seeds (WMS) with peanuts which was used for comparison purpose.
Results: The proximate screening indicated that protein (15.72%) and carbohydrate content (52.49%) was high in WMSNB compared to PNB. The prepared WMSNB showed rich in phenolics and also possessed good antioxidant activity against DPPH. The free fatty acid composition

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showed that linolenic acid, stearic acid and lauric acid of oil extracted from WMSNB were 51.15%, 10.65% and 6.49% respectively, while oleic and palmitic acid extracted from PNB were 38.11% and 14.62% respectively. The overall sensory evaluation showed that there was no significant difference (P<0.05) between WMSNB and PNB.
Conclusion: The antioxidant capacity of the WMSNB constituent underlines the potential source of natural antioxidants and bioactive compounds for the therapeutic purposes.

Keywords: Watermelon seeds; peanuts; seeds; antioxidant and nutribars.

ABBREVIATIONS

WMS : Watermelon Seeds
WMSNB : Watermelon Seed Nutribars
PNB : Peanut Nutribars

1. INTRODUCTION

Recently, there has been a greater emphasis on recovering useful components from neglected food portions and recycling them within the food chain in an economic and ecological manner [1]. Conversion of food waste is gaining popularity due to the fact that these materials represent potential usage sources for conversion into usable goods, as well as an increase in demand for natural bioactive components [2].

Watermelon (*Citrullus lantus)* is widely consumed around the world, and its seeds and rind are often discarded and used as animal feed [2]. These by-products contain phytochemical substances that have both nutritional and functional value. Watermelon seeds (WMS) are abundant in protein, containing amino acids such as tryptophan, glutamic acid, and lysine [3]. Existing data implies that WMS proteins have good *in-vitro* digestibility with few anti-nutritional factors, the seeds contain a reasonable amount of micronutrients, which have good biological function in human body. Due to their amino acid composition (high arginine content) they have therapeutic properties. Further seeds also have low carbohydrates but high in calories [4].

Roasted WMS can be an excellent alternative for the development of highly nutritious food products [4]. The nutrient part of water melon seeds or powder form can be used as additives in the food viz., flour, soup and nutribars etc [5].

Nutribars are energy-dense snacks made from cereals and other high-energy foods. Granola bars (marketed as a healthier alternative to candy bars), breakfast or cereal or snack bars (used as a meal substitute), and energy bars dominate the market [6]. People schedules are becoming busier as the world changes, necessitating increasing access to quick and easy food, particularly enticing energy bars. Currently, in the market nutribars are either pricey or employ traditional ingredients. As a result, there is a demand for a low-cost, high-nutritional-value nutribars that incorporates novel ingredients [7].

Watermelon seed/oil is an underrated product that contains essential fatty acids, vitamin E, minerals, and anti-oxidant activity, as well as being ideal for cooking [8]. The focus of this research is to raise public awareness about the qualities of WMS and its various benefits. Study reported that it has a good impact on growth and has cardioprotective, hepatoprotective, and anti-diabetic properties [9]. It is indicated that it is the demand of time to study its commercial potential after a complete toxicological investigation, animal studies, physicochemical properties, and nutritional analysis [9]. There is also limited literature on the nutritional, phytochemical and antioxidant properties of the WMS. With this background, the present study was to make high protein nutribars enriched with WMS for the sake of health benefits under low cost. The WMS is used as a novel ingredient to provide high protein along with jaggery, dry coconut, sesame seeds and ghee which also contribute to the dietary content and flavour profile of the desired product.

2. MATERIALS AND METHODS

Watermelon seeds (*Citrullus lanatus),* Dry coconut (*Cocos nucifera*), Sesame seeds (*Sesamum indicum*) and jaggery were procured from a local market in Kolar, Karnataka, India. Peanuts were purchased from the same market. The laboratory and analytical grade chemicals were purchased from chemical supplier from Hi-media, and Sisco research laboratories.

2.1 Preparation of Nutribars

Watermelon seeds (WMS) were roasted in a medium flame for 5-7 minutes in a pan and

allowed to cool to room temperature. Jaggery (50 grams) was added with minimum quantity of potable water and heated to make thick syrup. The heating of the syrup was continued till the temperature of the syrup reaches 115 °C. The roasted seeds, grated dry coconut and sesame seeds were then mixed with the hot jaggery syrup and poured into an aluminium plate, earlier smeared with sunflower oil. The product was spread uniformly and allowed to cool to room temperature. The cooled product was cut into 3cm × 3cm × 2 cm cuboids. For the peanut nutribars, all the ingredients remained the same expect, WMS was replaced with peanuts.

2.2 Proximate Analysis

The moisture, protein, crude fat contents, ash, crude fibre and total carbohydrates were carried out by AOAC (2004). The crude fat was estimated by exhaustive extraction with petroleum ether using a Soxhlet apparatus. The micro Kjeldahl method was used for the determination of protein (N × 6.25). The moisture, ash and crude fibre contents were determined by the AOAC (2004) method [10]. The total carbohydrate was obtained by difference of (100 – (% moisture + % crude protein + % crude fat + % ash).

2.3 Determinations of Total Phenol Content

Total phenols were extracted from a weighed portion (500 mg) of grinded nutribars samples with 5ml of 1.2 M HCl in 80 % aqueous methanol for 2 h and analysed by Folin-Ciocalteu micro method. Results are expressed as mg Gallic acid equivalent g^{-1} dry weight [11].

2.4 Determinations of Antioxidant Activity

The antioxidant activity was determined by means of DPPH radical scavenging assay [12]. To 0.2 mL of each extracted sample and the standard Trolox solutions, 3.8 mL of 0.1 mM DPPH solution was added in a test tube. The mixtures were shaken for 1 minute and then left in the dark for 30 minutes after which the absorbance was read using spectrophotometer at 517 nm against the blank. Absorbance of a control ($A_{control}$) was taken after adding DPPH radical solution to 0.2 mL of the extraction solvent (distilled water).

$$\% \text{ DPPH radical inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

From equation, the free radical scavenging (antioxidant) activity was expressed as the mean micromole of Trolox equivalent (µMTE/g).

2.5 Fatty Acid Composition

2.5.1 Oil extraction from the nutribars

Three hundred gram of developed nutribars was grinded into fine powdered and placed inside a thimble made from thick filter paper. This was then placed into the Soxhlet extractor's main chamber. Petroleum ether was utilised as the extraction solvent. For 5–10 hours, the solvent was heated to reflux at 100 °C. Following the extraction, the samples were placed in a fume hood for 1 hour to ensure that all of the petroleum ether in the crude oil was completely dried off [13].

2.5.2 Gas chromatography-mass spectrometric (GC-MS) analyses

The GC-MS analysis was carried out using Gas chromatograph interfaced to a mass spectrometer GC-MS-QP 2010 Plus Shimadzu system (GC-MS) employing the following conditions: Column Elite-1 fused silica capillary column (30 m x 0.25 mm 1D x µl df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (Split ratio of 10:1) injector temperature – 250 ^{o}C; ion-source temperature 208 ^{o}C. The oven temperature was programmed from 110 ^{o}C (Isothermal for 2 min) with an increase of 10 ^{o}C /min to 200 ^{0}C then 5 ^{o}C /min to 280 ^{o}C/min, ending with a 9 min isothermal at 280 ^{o}C. Mass spectra was taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram will be a turbomass.

2.5.3 Identification of components

The mass spectrum of GC-MS was interpreted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns by comparing the mass spectrum of the unknown components with those of the known elements stored in the NIST library.

The name, molecular weight and chemical structure of the test material were ascertained.

2.6 Sensory Quality Characteristics of Developed Nutribars Samples

Developed nutribars samples were subjected to sensory analysis based on 9-point hedonic scale as described by larmond (1997) [14]. It was tested for appearance, texture, flavour, taste, aroma and overall acceptability using an in-house trained panel of 5 members. Panel members were advised to use verbal descriptions and convert them into scores. The scores were based on the following criteria: Like extremely: 9; Like moderately: 7-8; like slightly: 5-6; dislike slightly: 3-4; and dislike extremely: 0-2. The scores were averaged.

2.7 Statistical Analysis

Data were subjected to analyse statistically with the aid of the Statistical Analysis Software by using SPSS.16 software.

3. RESULTS AND DISCUSSION

The formulation used for the preparation of WMNSB and PNB nutribars is depicted in the Table 1. Prepared nutribars from watermelon seeds and peanuts are shown in Fig. 1.

Table 1. Ingredients used to prepare nutribars

Ingredients	Quantity
Watermelon seeds*	100gms
Jaggery	50gms
Sesame seeds	10gms
Coconut flakes	15gms
Ghee	5gms

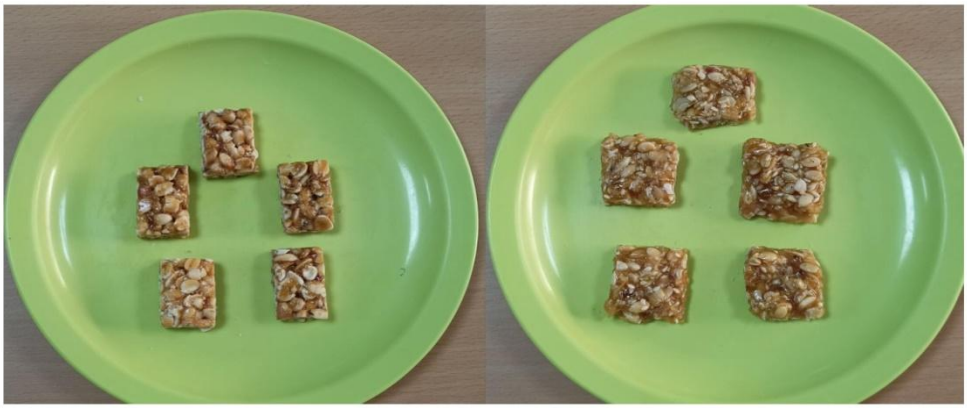
**For the peanut nutribars, all the ingredients remained the same expect, watermelon seeds was replaced with peanuts*

3.1 Proximate Analysis

The results of proximate composition are present in Table 2. WMSNB was found to have moisture 4.87%, 15.72% crude protein, 1.57% ash, 21.89% fat, 3.46% fibre, and 52.49% carbohydrate. On the other hand, PNB was found to have 5.33% moisture, 12.45% crude protein, 1.42% ash, 26.69% fat, 3.39% fibre and 50.37% carbohydrate content. The carbohydrate and protein content of WMSNB was found to be higher when compared to PNB. This observation can be explained by incorporation of watermelon seeds in the nutribars preparation which leads to increase in the protein content in WMSNB. The result of carbohydrate content was contradicting to the results reported by Virginia and Ajit (2004)[4]. They exhibited low carbohydrate content in watermelon seeds. This observation could possibly be due to changes in soil type, harvest time, regional differences, genotype, geographical and the environmental conditions in which watermelons are grown could all contribute to differences in carbohydrate content. Our results is also agreement with the report of Peter-Ikechukwu AI et al., (2018) who demonstrated that addition of toasted watermelon seeds in cookies enhanced the protein, fat, crude fibre, and ash content while decreasing the carbohydrate content as the substitution amount of wheat flour increased [15]. A study also reported that watermelon seeds are abundant in protein, containing amino acids such as tryptophan, glutamic acid, citrulline, arginine and lysine and also have good *in-vitro* digestibility which indicates that they have therapeutic properties [4]. Another study found that taking 120 mg/kg/day WMS extracts for three weeks reduced serum cholesterol, C-reactive proteins, glutathione, and catalase levels in adult male mice, perhaps due to the presence of citrulline and arginine in WMS extract [16].

3.2 Total Phenolic Content (TPC) and DPPH Activity

The antioxidant properties of the polyphenolics in the seeds suggest that they could serve as reductones, donating electrons to free radicals and inhibiting free radical-mediated chain events [17]. The result of TPC and DPPH activity is depicted in Table 3. In the present study the TPC of WMSNB (7.90 mg GAE/g) was found to be highest than PNB (4.28 mg GAE/g). TPC content was shown to indicate the level of DPPH radical scavenging activity in a sample, and samples high in phenolic content also have more DPPH inhibition [18]. This trend was observed in the present study where WMSNB exhibited the highest TPC and also exhibited highest percentage of DPPH inhibition (63%) when compared with PNB (46%). Tabiri et al.,(2016) reported that the seeds from the crimson sweet variety contained the highest amount of phenols with a value of 54.16 mg GAE/g followed by Black diamond variety and Charleston gray variety [19]. A study reported that methanolic extract of WMS contains phytochemical components like phenols, flavonoids, saponins, and terpenoids glycosides which have strong



Peanut nutribar Watermelon Seed nutribar

Fig. 1. Photograph of prepared nutribars

Table 2. The proximate composition of watermelon seed nutribars and peanut nutribars

Sample	Moisture (%)	Protein (%)	Total ash (%)	Total fat (%)	Crude fibre (%)	Total carbohydrate (%)
WMSNB	4.87± 1.63[b]	15.72± 1.88[a]	1.57± 0.33[b]	21.89±1.33[b]	3.46± 0.88[a]	52.49 ± 1.89[a]
PNB	5.33± 1.13[a]	12.45± 1.45[b]	1.77± 0.13[a]	26.69±1.89[a]	3.39± 0.48[b]	50.37± 0.53[b]

Values not sharing a common superscript letter in a column are significantly different at (P<0.05). PNB-Peanut nutribars, WMSNB-Watermelon seed nutribars.

antioxidant activity, which protects cells from the damaging effects of ROS such as superoxide, hydroxyl and peroxyl radicals, and single oxygen, all of which contribute to oxidative stress [20].

Table 3. Total phenolic content and antioxidant activities of nutribars samples

Sample	Total phenolic content [mg GAE/g]	DPPH inhibition (%)
WMSNB	7.90± 1.43[a]	63± 1.83[a]
PNB	4.28± 1.83[b]	46± 2.43[b]

Values not sharing a common superscript letter in a column are significantly different at (P<0.05). PNB-Peanut nutribars, WMSNB-Watermelon seed nutribars.

3.3 Free Fatty Acid Composition

Table 4 presents the free fatty acids identified in the WMSNB and PNB. It was found that WMSNB were rich source of linoleic acid (51.15%), stearic acid (10.65%) and lauric acid (6.49%) when compared to PNB. In humans, Alpha-Linolenic acid (ALA) is an essential fatty acid because it cannot be synthesized from saturated fatty acids, n-9 monounsaturated fatty acids, or n-6 polyunsaturated fatty acids (PUFAs). So, ALA should be obtained from diet through foods and snacks [21]. In the present study, the free fatty acid profile indicates that WMSNB have high level of linoleic acid. It has been reported that consuming the watermelon seed oil will reduce the risk of cardiovascular heart disease [22]. A researcher reported that after going through toxicological evaluation, nutritional and physiological benefit, WMS oil exhibited safety within the recommended ranges. Hence it might be an excellent cooking and frying oil. Therefore WMS oil should be commercially exploited to be used in different nutraceuticals and functional food commodities, as well as a potential antidote for fighting against various ailments [9]. In view of these farmers should be encouraged to plant more of these melon so that seeds are available in bulk to industries for oil extraction, similar to how soya beans and groundnut seeds are used for extraction of oil and sold for human use.

3.4 Sensory Evaluation

The sensory quality parameters, namely, appearance, texture, flavour, taste, aroma and overall acceptability of the both nutribars values are listed in the (Table 5). It was observed that the panellist could not find any significant

Table 4. The free fatty acid composition of nutribars samples

Sl. No	Parameter	PNB	WMSNB
1	Linolenic acid (C18:2n6c)	32.36	51.15
2	Oleic acid (C18:1n9c)	38.11	13.14
3	Palmitic acid (C16:0)	14.62	12.81
4	Stearic acid (C18:0)	4.88	10.65
5	Behenic acid (C22:0)	3.37	ND
6	Lauric acid(C12:0)	0.35	6.49
7	Myristic acid (C 14:0)	0.18	2.65
8	Linolenic acid(C18:3n3)	1.16	0.08
9	Caprylic acid (C10:0)	0.05	1.14
10	Lignoceric acid (C24:0)	1.05	ND
11	Tricosanoic acid (C23:0)	0.81	0.81
12	Capric acid (C10:0)	ND	0.81
13	Cis-11-Eicosenoic acid(C20:2)	ND	0.17
14	Palmitoic acid (C16:1)	0.08	0.09
15	Caproic acid (C4:0)	ND	0.09
16	Heptadeconic acid (C17:0)	ND	0.05

PNB-Peanut nutribars, WMSNB-Watermelon seed nutribars.

Table 5. Sensory evaluation of nutribars prepared from peanut and watermelon seeds

Nutribars	Appearance	Texture	Flavour	Taste	Aroma	Overall acceptability
WMSNB	8.62±0.3[a]	7.58±0.43[a]	8.00±0.20[a]	8.6±0.12[a]	8.2±0.37[a]	8.5±0.18[a]
PNB	8.55±0.3[a]	7.49±0.42[a]	8.12±0.19[a]	8.5±0.10[a]	8.1±0.34[a]	8.2±0.19[a]

Values not sharing a common superscript letter in a column are significantly different at (P<0.05).
PNB-Peanut nutribars, WMSNB-Watermelon seed nutribars

difference (*P*<0.05) between PNB and WMSNB in terms of appearance, texture, flavour, taste and overall acceptability. This is consistent with the results obtained by Ubbor & Akobundu (2009) showed that when bread fortified with watermelon seed flour did not show any significant difference in sensory attributes [23]. Although, in this study, the WMSNB sample scored slightly higher acceptability in terms of organoleptic quality when compared to the PNB.

4. CONCLUSION

Watermelon fruit is widely consumed around the world; however it contains a large number of seeds that are discarded. These seeds are rich in protein content and also contain phytochemical substances that have both nutritional and functional value. In this context, the present study was aimed to develop high protein nutribars enriched with watermelon seeds for the sake of human health benefits properties under low cost. This research paves the way for greater utilisation and value addition of WMS grown in different countries. Farmers who grow these watermelon fruits will benefit from the popularisation and commercialization of the developed products. In this study, the proximate screening indicated that protein and carbohydrate content was high in WMSNB and also showed rich in phenolic content which possess good antioxidant activity against DPPH. Further, the oil extracted from WMSNB showed the presence of linolenic acid, stearic acid and lauric acid which are reported to have biological activities that influence the metabolism, function, and responsiveness of cells and tissues to hormones and other signals. However, more clinical research on water melon seed is needed to effectively support the development of functional food products, nutraceutical, and pharmaceutical uses.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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