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THE QUALITY OF FRUIT BARS AND CRACKERS DESIGNATED FOR THE RAW FOOD VEGAN DIET

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Fruit bars and crackers designated for the raw vegan diet were evaluated with regard to the microbial quality, the presence of antioxidants (phenolics, flavonoids, and condensed tannins), and antioxidant properties. Since these products had been subjected to air drying up at 42 °C, the oxidation state of the fat (peroxide and thiobarbituric values) and the activity of superoxide dismutase and catalase was determined. In general, the samples were considered to be of good microbial quality, high antioxidant content, and capacity. As found out, the drying process did not alter the lipid oxidation and kept the activity of catalase constant in finished products.

Introduction

There is an increasing demand for the alternative nutritional guidelines within the two decades in the Czech Republic. Among them, a raw food vegan diet has

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become the most popular representing a consumption of raw uncooked meal. In the Czech Republic, a specific phrase “life food” has been established, and the people practicing the consumption of such raw uncooked meal call themselves “vitarians”. Fresh fruits and vegetables, germinated seeds, cereals, and nuts are the main components of their daily diet. They believe that the foods in its natural origin, i.e. without temperature treatment have significantly higher nutritive value in comparison with food exposed to the high temperature treatment. Since the fresh food products can easily decay during storage from both microbiological and chemical point of views, it is allowed to apply a mild temperature treatment. It usually comprises the drying of fresh products up to 48 °C [1]; however, the maximal temperature threshold has never been unified in literature and one can find it reported to be in the range from 38 °C to 48 °C. The raw food diet proponents claim that this temperature keeps the enzymes intact and leads to a better digestion; however it has not been supported by any relevant study. Scarcely, the respective data about the effect of the raw food vegan diet on the human health are available. Vegan diet seemed to lower the risk for overall and female specific cancer [2,3], when decreasing the mortality in comparison with omnivores [4]. It was also confirmed by Ling and Hänninen [5] that one-week consumption of raw food vegan diet significantly decreased the activity of some pro-carcinogenic enzymes formed by the intestinal microbiota. In addition, the effect of vegan diet on the content of gut microbiota has been reviewed exhibiting the protective effect [6,7]. The risk for the human health associated with the consumption of strict vegan diet (including raw food) has also been described. A low intake of vitamin B12 [8], and the decrease of triacylglycerole and cholesterol content in plasma (even HDL) can promote severe health damage [9]. In an extensive study, the dramatic decrease of the body weight of both male and female patients was documented together with some underweight-related health complications [10].

The main objective of this study was to determine the quality of fruit bars and crackers commercially available on the market. These products are declared to fulfil the specification for raw vegan food, i.e. a minimal temperature treatment.

Materials and Methods

Samples

Eight samples, containing four types of fruit bars (FB1-4) and four types of crackers (C1-4) were obtained from a local company declaring their products as being suitable for “vitarians”. The products were manufactured according to the Council regulation (ES) No. 834/2007 on organic production and labelling organic products, air-dried up to 42 °C, and kept under modified atmosphere packages. The ingredients used for the manufacturing are listed in Table I.

Table I The ingredients of the fruit bars and crackers

Samples		Ingredients
Fruit bars	FB1	Date fruits, almonds, cashew, dried cherries, baobab powder, lyophilised powder of cranberry and maca (a herbaceous plant, 2% soln.), salt
	FB2	Date fruits, almonds, nuts (Brazil, pistachio, pecan), seeds (chia, sesame), coconut, dried juice from young barley, coconut virgin oil, vanilla
	FB3	Date fruits, almonds, hemp protein powder, pumpkin seeds, almonds paste, chocolate (32% of cacao butter), coconut virgin oil, spirulina powder, vanilla
	FB4	Date fruits, dried cherry, cashew nuts, almonds
Crackers	C1	Sunflower seeds, linseed, carrots, salt
	C2	Sauerkraut, almonds, seeds (pumpkin, linseed, hemp, chia), salt and cumin
	C3	Cashew nuts, hemp seed, carrot, onion, dried tomato and dill, salt and condiments
	C4	Linseed, sunflower seed, dried tomato, salt, condiments

Microbiological Analysis

The following nutrient media (HiMedia Laboratories Pvt. Ltd., India) were applied to a specific microorganism quantification: Total viable count (PCA) for mesophilic aerobic and facultative anaerobic microorganisms; a dichloran medium base with rose bengal for total yeast (DRBC-y) and fungi (DRBC-f) count; a violet red-bile agar (VRBA) for total coliform bacteria count, and peptone glucose with *Bromcresol Purpur* (PBKP) for aerobic spore-forming bacteria count. In addition, the presence of sulphite-reducing clostridia (SRC) was determined using sulphite agar with ferrous sulphate. The package was aseptically opened, and 10.0 g sample homogenized in a plastic bag with 90 ml of physiological saline. An 1.0 ml and 0.1 ml aliquots were transferred onto the appropriate agar plates and incubated in the following way: 30 °C and 24-48 h for PCA count, 25 °C and 6-7 days for DRBC count, 30 °C and 24-48 h for VRBA, and 37 °C for 24 h after inactivation of living cells at 90 °C (10 min) in the case of PBKP count. The presence of SRC was determined in tubes after inactivation of living cells (90 °C, 10 min) and the subsequent incubation at 37 °C for 6-7 days. The experiment was repeated in two separate trials; each being cultivated in two agar plates ($n = 4$). The results were expressed as the log(CFU g^{-1}).

Chemical Analysis

One gram of the sample was extracted in 50% methanol solution for 30 min in sonication bath followed by the filtration of solid particles. The extract was used in further analysis. The content of total phenolics was determined by Folin-Ciocalteau assay adopted from Pasha *et al*. [11]. A 1.0 ml of sample solution was added to test tube together with 1.0 ml of 96% ethanol, 5.0 ml of distilled water and 0.5 ml of Folin-Ciocalteau reagent solution. The mixture was allowed to stand for 5 min, then 1.0 ml of 5% Na_2CO_3 was added. After 70 min standing in dark cabinet, the absorbance of the mixture was measured at 765 nm. The results were expressed as gallic acid equivalents.

The content of flavonoids in the extract was measured by the $AlCl_3$ method, which is based on the formation of flavonoid-aluminium complex [12]. The absorbance was observed at 415 nm, and the results were expressed as quercetin equivalent. The content of condensed tannins of the extract was determined according to the following procedure [13]: a 1.0 ml of the extract was mixed with 2.0 ml of vanillin solution (1% in 7.0 M H_2SO_4), and the absorbance was measured after 25 min at 500 nm. The results were expressed as catechin equivalents. Antioxidant capacity was measured by suppressing the activity of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) [14], and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) [15] free radicals using Trolox as the standard antioxidant.

For the purpose of the oxidation state analysis of the samples, the fat was extracted using diethylether containing 7.0 ppm of butylated hydroxytoluene (BHT) in the Soxhlet apparatus. BHT was added in order to prevent the oxidation of fat during extraction. The fat extracted was examined for the content of primary (peroxide value, PV) and secondary oxidation products (thiobarbituric value, TBAR) [16,17].

The activity of catalase (CAT) of ethanol extracts was determined according to Mehnaz *et al*. [18]. For the CAT assay, hydrogen peroxide was used as a substrate when the reaction kinetics being detected at 240 nm (molar extinction coefficient $\varepsilon = 34.91\ l\ mol^{-1}\ cm^{-1}$). Helios Delta and Gamma spectrophotometers (Thermo Spectronic, Thermo Scientific, USA) have been used for all the experiments.

The results of the chemical analysis represent the average values of two replicates in two separate trials ($n = 4$). The pairwise comparison (Spearman coefficient) was used for the determination of significant differences among samples, and an attempt to find the correlation coefficients between the variables was made with the probability level $p = 0.05$ (OriginPro 9.0, OriginLab Corp., MA, USA).

Results and Discussion

Microbiological Analysis

The results of microbiological analysis are presented in Table II. In this study, the PCA count ranged from log 2.68 to log 4.34 CFU g^{-1} indicating the good microbial quality of the products tested. Although there is no hygienic limit for such a kind of food products in the current legislation, log 6 CFU g^{-1} is generally considered as the safe limit. Yeast viable counts (DRBC-y) ranged from log 1.36 to log 3.70 CFU g^{-1} with exception of FB4 where no yeasts had been detected.

Fungi were found in all the samples in the range of log 1.83-log 3.67 CFU g^{-1}. A great variability of coliform bacterial counts (VRBA) was determined with a significantly higher value for FB4 sample ($p < 0.01$) and with no viable count detected in FB2 and C3 samples. In FB2 sample, dried juice from young barley may exhibits higher antimicrobial activity as previously reported in the study of McClean *et al*. 2014 [19].

Table II Microbial quality of fruit bars (FB1-FB4) and crackers (C1-C4)

Sample	PCA[1]	DRBC-y[2]	DRBC-f[3]	VRBA[4]	BPPK[5]
	log (CFU g^{-1})				
FB1	$^{b}3.6 \pm 0.3$	$^{b}3.3 \pm 0.4$	$^{d}3.4 \pm 0.1$	$^{a}2.3 \pm 0.3$	$^{a}1.9 \pm 0.4$
FB2	$^{b}3.4 \pm 0.3$	$^{a}2.0 \pm 0.2$	$^{d}3.3 \pm 0.1$	> 1.0	$^{a}1.9 \pm 0.4$
FB3	$^{b}3.9 \pm 0.2$	$^{a}2.2 \pm 0.4$	$^{f}3.7 \pm 0.1$	$^{a}2.8 \pm 0.4$	$^{b}3.5 \pm 0.2$
FB4	$^{a}2.7 \pm 0.1$	> 1.0	$^{b}2.9 \pm 0.1$	$^{b}4.4 \pm 0.5$	$^{ab}2.8 \pm 0.8$
C1	$^{bc}3.9 \pm 0.4$	$^{b}3.2 \pm 0.2$	$^{de}3.2 \pm 0.1$	$^{b}3.6 \pm 0.3$	$^{a}2.0 \pm 0.1$
C2	$^{b}3.3 \pm 0.1$	$^{a}1.7 \pm 0.2$	$^{bce}2.8 \pm 0.3$	$^{a}1.9 \pm 0.3$	$^{a}1.7 \pm 0.1$
C3	$^{c}4.3 \pm 0.1$	$^{b}3.6 \pm 0.4$	$^{c}2.5 \pm 0.1$	> 1.0	$^{a}1.8 \pm 0.2$
C4	$^{b}3.5 \pm 0.2$	$^{a}2.8 \pm 0.6$	$^{a}2.1 \pm 0.2$	$^{a}2.4 \pm 0.7$	$^{a}2.3 \pm 0.2$

Total viable count (1), yeast (2), fungi (3), coliform bacteria (4), and aerobic spore-forming bacteria (5) in replicates ($n = 4$). Different letters (a-f) in superscript indicate significant differences in column

The aerobic spore-forming bacteria (PBKP) were also detected in all the samples being significantly higher in fruit bars sample compared to crackers ($p < 0.05$). Anaerobic spore-forming bacteria (SRC) were detected only in fruit bar samples (FB1-FB4). The fruit bars provided favourable conditions for the survival of both aerobic and anaerobic spore-forming bacteria. Fruit bars are thick (15 mm in height) whereas crackers are porous and thin products (2-3 mm).

Table III The content of various antioxidants and appropriate antioxidant capacity of fruit bars and crackers. Average mean ± standard deviation of replicates (n = 4)

Sample	Total fenolics[1]	Flavonoids[2]	Condensed tannins[3]	DPPH[4]	ABTS[4]
		mg/100 g d.b.		mg/g d.b.	
FB1	^{a}115.0 ± 17.0	$^{d, g}$41.0 ± 7.0	^{c}22.9 ± 0.5	^{a}1.81 ± 0.30	^{a}24.47 ± 0.74
FB2	^{b}225.0 ± 12.0	^{a}6.0 ± 1.0	^{d}54.0 ± 2.0	^{b}3.85 ± 0.12	^{a}37.13 ± 0.62
FB3	^{b}219.0 ± 22.0	^{d,f,h}42.0 ± 3.0	^{b}36.0 ± 8.0	^{b}3.46 ± 0.16	^{b}114.0 ± 5.6
FB4	^{b}241.0 ± 19.0	^{d,f,h}42.0 ± 11.0	^{b}36.0 ± 5.0	^{c}5.04 ± 0.23	^{b}111.8 ± 6.6
C1	^{c}397.0 ± 8.0	^{e,f}55.0 ± 5.0	^{a}8.8 ± 0.3	^{d}20.21 ± 0.74	^{d}137.8 ± 10.4
C2	^{a}156.0 ± 4.0	^{b,c,g}28.0 ± 6.0	^{a,c}13.9 ± 0.9	^{b}3.08 ± 0.07	^{a}16.6 ± 5.6
C3	^{a}144.0 ± 44.0	^{a,b}18.0 ± 5.0	^{a}6.1 ± 0.9	^{a}1.70 ± 0.30	^{c}74.3 ± 9.9
C4	^{b}222.0 ± 9.0	^{a,c}20.0 ± 1.0	^{a}6.2 ± 0.3	^{c}4.90 ± 0.15	^{d}139.0 ± 9.4

Expressed as gallic acid (1), quercetin (2), catechin (3) and Trolox (4) equivalents. Different characters (a-g) in superscript indicate the significant differences in the respective column

Chemical Analysis

As indicated in Table III, high content of total phenolic compounds was determined in all the samples; especially, those contained in fruits or vegetables. Among the samples involved in this study, C1 had the highest level of phenolic compounds and flavonoids, whereas significantly higher content of condensed tannins and lower-to-moderate content of phenolics and flavonoids was determined in FB2 sample. Cracker samples (C1) contain 13 % (w/w) of carrots, which seems to be the main contributor to the high phenolic content as found in other studies [20,21]. The high amount of condensed tannins in FB2 sample can be explained by the presence of nuts and seeds as the main ingredients (see Table I). In literature, the nut oils are the major source of condensed tannins [22]. The sample with carrots also displayed ten-fold higher antioxidant capacity in comparison with other samples in this study, as determined by DPPH assay. It was found out that carrots are also rich in the antocyanins content causing a very high antiradical activity [20]. The crackers with carrots (C1 sample) also possessed the highest antioxidant capacity measured by ABTS assay (137.8 mg Trolox g^{-1} d. b.) followed by the sample C4 (139.0 mg Trolox g^{-1} d. b.) which contained 9 % (w/w) of dried tomatoes. The antioxidant properties of tomatoes were studied with respect to the various drying technologies and the authors of this study reported on a significant increase of antioxidant activity in comparison with fresh

tomatoes [23].

The peroxide values (PV) were determined in the range from 0.31 to 0.64 meqv O_2 kg^{-1} and TBARs of the extracted fats were found to be within the range from 0.52 to 9.73 MDAeqv g^{-1} (see Table IV).

Table IV The quality of oil extracted from fruit bars and crackers . Average mean ± standard deviation of replicates (n = 4)

Sample	Peroxide value[1]	TBAR value[2]
FB1	^{a}0.41 ± 0.08	^{a}0.52 ± 0.09
FB2	^{ab}0.49 ± 0.03	^{a}0.70 ± 0.05
FB3	^{a}0.31 ± 0.09	^{a}0.73 ± 0.03
FB4	^{ab}0.53 ± 0.19	^{a}1.03 ± 0.16
C1	^{b}0.60 ± 0.21	^{c}9.73 ± 0.69
C2	^{b}0.60 ± 0.09	^{b}1.63 ± 0.06
C3	^{b}0.64 ± 0.03	^{b}3.74 ± 0.26
C4	^{b}0.70 ± 0.05	^{a}0.83 ± 0.03

Expressed as meqv. O_2 kg^{-1} (1) and MDAeqv. g^{-1} (2). Different letters (a-c) in superscript indicate significant differences in column

While very low PV indicates good quality of the extracted oil, a relatively high value of TBAR for S1 sample can be caused by interfering compounds that are present in carrots.

In the fruit bars and crackers used for raw vegan food diet, the enzymatic activity is the basic principle of "vitarian's" philosophy. Although the benefits of the consumption of food with active enzymes on the human health have not been examined into detail, the presence of enzymes as such indicates that the low temperature was applied during the processing.

Catalase was active in all the samples ranged from 7.48 ± 0.02 to 20.88 ± 0.62 U mg^{-1}.

It has already been described that the microorganisms we are consuming had significantly influenced the gut microbiota [7]. The same authors also pointed out that the composition of meal had correlated with the presence of particular group of microorganisms. In our study, we have found that coliform bacteria counts correlated only with the DPPH activity (r = 0.766, $p < 0.05$) and the flavonoid content (r = 0.826, $p < 0.05$).

Conclusion

All the products were rich in phenolic compounds, flavonoids, and condensed tannins when having also expressed the high antioxidant capacity ascertained by means of DPPH and ABTS assays. Crackers with carrots exhibited the highest antioxidant activity among the samples. Despite the fact that low temperature treatment was used during manufacturing, the overall microbial quality was found appropriate. Aerobic spore-forming bacteria count was significantly higher in fruit bars than that in crackers, and additionally, contained anaerobic spore-forming bacteria as well. The oxidative state of fats extracted from the samples was of good quality, indicating also the proper selection of the ingredients by the manufacturer.

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