Characterization of the kefir beverage produced from yam (*Colocasia esculenta* L.), sesame seed (*Sesamum indicum* L.) and bean (*Phaseolus vulgaris* L.) extracts

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Abstract The objective of this study was to evaluate the characteristics of kefir beverages made from yam, sesame and bean extracts after fermentation of the extracts with water kefir grains. Formulations were prepared with raw and boiled yams. To the yam extract, sesame and different percentages of white bean extract were added. The fermentation kinetics, the chemical composition and color of the extracts were analyzed. To determine the fermentation kinetic parameters, pH and titratable acidity were evaluated. The chemical composition and color of all extracts were determined before and after fermentation process. The decrease in pH and increase in titratable acidity were affected both by raw or boiler yam. The addition of bean extract favored the decrease in pH of formulations containing extracts of raw or boiled yams. Chemical composition and color of the beverages were altered after fermentation. The kefir beverage made from yam and sesame enriched with 50% beans proved to be an excellent fermentation substrate. In addition, it is a new way of consuming vegetable products, especially fermented products, which have limited diversity to serve vegan consumers and also people with certain allergies to dairy products.

Keyword Kefir · Fermentation · Plant extracts · Functional foods

Introduction

In recent years, consumers have shown great interest in consuming foods that can prevent diseases. Consequently, functional food research has attracted the interest of several researchers to increase the diversity of these products on the market.

In general, fermented milk is the main protein matrix for binding of probiotic microorganisms consumed worldwide (Oliveira 2009). However, a small portion of the population has allergy to dairy products, and this deprives these consumers of the health benefits which stem from probiotic food consumption. Additionally, the number of people who adopt vegan vegetarianism has increased every day. To meet the demand of part of this population with dietary restrictions, the development of non-dairy probiotic products has been the main focus of some researches. Fruit juices, desserts and cereal products have been considered excellent progeny-bearing matrices (Reichert 2008).

The high cost of probiotic products is a factor that makes it impossible for their acquisition by the low-income population. However, the consumption of kefir beverages has been a healthy alternative, more accessible to this population. This is made possible because kefir drinks are prepared in the home environment.

Kefir grains are starter cultures used to produce the kefir beverage, which is obtained by fermenting milk or another desirable substrate with kefir grains. These grains contain a complex symbiotic community of lactic bacteria (*Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*), yeasts (*Kluyveromyces*, *Saccharomyces* and

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Torula) and acetic bacteria (*Acetobacter*) confined in a matrix of polysaccharides and proteins (Farnworth 2005). Furthermore, lactic acid bacteria with probiotic potential have been isolated in some kefir beverages (Leite et al. 2015). In view of those findings, kefir has been recognized as a functional food capable of promoting health benefits for consumers.

Milk kefir is considered the most traditional fermented drink, followed by water kefir supplemented with brown sugar (John and Deeseenthum 2015). However, nowadays alternative kefir beverages have been proposed, replacing milk or brown sugar with other carbohydrate sources such as cocoa (Puerari et al. 2012) and fruit juices (Corona et al. 2016; Randazzo et al. 2016). Still, the non-dairy kefir drinks currently developed are not sources of protein. Thus, the development of fermented kefir beverages from plant extracts, rich in carbohydrates and proteins, may be a healthier option for the population with dietary restrictions.

The yam may be considered an alternative in the preparation of vegetable-based drinks, due to its high content of carbohydrates in addition to its neutral taste and odor (Miranda 2008). However, the yam is a poor source of protein. Beans are legumes which have a protein content ranging from 18.0 to 25.0%, and therefore comprise an excellent source of protein to be incorporated into vegetable drinks. Yams and beans have a low lipid content, and the reduced concentration of this biomolecule in vegetable drinks might taint the achievement of a more homogenous mixture. A healthy alternative is the use of sesame, which has a high lipid content, and consequently may have an emulsifying role in the mixture. The use of different milk substrates in the elaboration of milk kefir or water kefir with brown sugar is not reported in scientific literature. This may be justified by the fact that kefir is prepared in the domestic environment, which implies the scarcity of scientific information, such as quantification of nutrients, chemical composition and products from microbial fermentation. However, when one desires to make a fermented beverage it is essential to assess if the new substrate will adversely affect the fermentation process initiated by lactic bacteria or yeast. Thus, the objective of this study was to evaluate the characteristics of kefir beverages made from yam, sesame and bean extracts after fermentation of the extracts with water kefir grains.

Materials and methods

Ingredients and preparation of plant extracts

The vegetable drinks were obtained from yams (*Colocasia esculenta* L.), white beans (*Phaseolus vulgaris* L.) and sesame (*Seasamun indicum* L.), according to Fig. 1. The

products were purchased at the local market in Brasília, Distrito Federal, Brazil. The yam was pre-washed with mineral water and peeled before processing. The extracts of raw or boiled yam were used as base substrates in elaboration of the drinks, thus developing two base formulations.

Raw yam extracts with sesame addition 100 g of sesame were soaked in water for 12 h. After this time, the hydrated sesame was mixed with 200 g of raw yam in 1 L of water. The mixture was ground in a blender for 3 min, and it was then strained and the obtained residue removed.

Cooked yam extract with sesame 200 g of yam were cooked in an opened system under atmospheric pressure in 1 L of water for approximately 1 h, the time needed to evaporate the added water. The same procedure described above was used for sesame. Then, 1 L of water was added to the cooked yam blend along with the sesame and this mixture was ground for 3 min in a blender. The mixture was then strained and the residue removed.

Bean Extract 200 g of white beans were soaked in water for 12 h. After this time the shells surrounding all the grains were manually removed. Then 1 L liter of water was added and the beans were cooked in an opened system under atmospheric pressure for 1 h. This cooking time was required to evaporate the added water. Finally, 1 L of water was added and the mixture was ground in a blender for 3 min.

The formulations elaborated were as follows: (1) yam extract with sesame; (2) bean extract; (3) yam extract with sesame with 5.0% brown sugar; (4) 75.0% yam extract with sesame + 25.0% bean extract with 5.0% brown sugar; (5) 50.0% yam extract with sesame + 50.0% bean extract with 5.0% brown sugar.

Preparation of the kefir beverage and determination of fermentation kinetic parameters

The formulations of extracts iii, iv and v were inoculated with 5.0% water kefir culture, with addition of 5.0% brown sugar. The mixture was homogenized and then incubated at 22 °C in an incubator (Model MA 415, Marconi, Piracicaba, Brazil) for 24 h. The kefir grains were obtained by donation in Brasília, Distrito Federal, Brazil. To determine the fermentation kinetic parameters, pH and titratable acidity were evaluated immediately after inoculation, and after 12 h of fermentation, every three hours, for up to 24 h. Previous evaluations indicated inexpressive pH and titratable acidity variation before 12 h of fermentation. The pH was determined using a potentiometer (Digimed, model DM21). The titratable acidity was determined by the method described by the Adolf Lutz Institute (2008), with the results expressed in mL of 1 mol L⁻¹ NaOH per 100 g.

Fig. 1 Scheme for obtaining kefir beverages

Determination of chemical composition

The chemical composition of all extracts was determined before and after the fermentation process. Moisture determination was performed according to the Analytical Standards of the Adolfo Lutz Institute (2008). The protein content of the vegetable drinks was assessed according to AOAC (2005), method 991.22. The total lipid content was determined by extraction method Am 5-04, using Ankom Technology's XT15 Extractor (AOCS 2005). Determination of the ash content in the samples was performed by the method 945.45 (AOAC 2005). The total carbohydrates content present in the products was determined as the difference, subtracting from 100 the values found for protein content, lipid content and ash content, according to method 986.25 (AOAC 2005). The protein content, lipid content, ash content and carbohydrate content were expressed as dry basis.

Determination of color

Color evaluation was performed with the aid of the ColorQuest XE Spectrophotometer (HunterLab, Reston, United States), obtaining values for the coordinates L, a and b of the Hunter system. With the values of the coordinates a and b, the hue angle (h° , Eq. 1) and color saturation or

chroma (C, Eq. 2) were obtained (Francis 1975; Mclellan et al. 1995).

$$h^\circ = \arctan(b/a) \quad (1)$$

$$C = \sqrt{a^2 + b^2} \quad (2)$$

where L = measurable in terms of white to black intensity; a = measurable in terms of red and green intensity; and b = measurable in terms of yellow and blue intensity.

Statistical analysis

A completely randomized design was adopted, with three replicates. Initially, analysis of variance was performed at 5% probability. Afterwards, a regression analysis was performed for the fermentation kinetics parameters and Tukey's test for the variables related to the composition and color of the extracts. For the analysis of variance and Tukey's test, we used the program Assistat 7.5 (Silva and Azevedo 2009). SigmaPlot software version 10.0 (Systat 2006) was used to obtain the equations and plot the graphs.

Results and discussion

pH and titratable acidity

A significant decrease in pH was observed in all beverages made with vegetable extracts to which water kefir grains were added (Fig. 2, Table 1 in the supplementary material). It was found that the formulation containing 50% raw yam extract with sesame, enriched with 50% of bean extract, was that which reached the lowest pH value after 24 h of fermentation (Fig. 2a). The estimated pH variation obtained from the regression equations (Table 1 on the electronic supplementary material) for the formulation containing raw yams and sesame, enriched with 50.0% bean extract, was 1.83. In the formulation without addition of the bean extract the estimated pH variation was 0.95. When boiled yam was used in preparation of the formulations, the same trend of decreasing pH was observed

Fig. 2 Regression curves of pH of extracts containing raw yams (a) and cooked yams (b), sesame and beans inoculated with water kefir grains for 24 h. RY raw yam, SE sesame, WB white beans, CY cooked yam

(Fig. 2b). Variations in pH equals to 1.27 and 1.66 were obtained in the formulations containing only yam extract cooked with sesame and in that enriched with 50.0% bean extract, respectively. Thus, the addition of bean extract favored the decrease in pH of formulations containing extracts of raw or boiled yams. However, there was a more pronounced effect on the pH decrease when raw yams were used. The formulation containing raw yams enriched with 50.0% beans presented a pH variation 92.6% higher than that obtained for the formulation without bean extract. When cooked yams were used, there was an increase in pH variation of 30.7.

With regards to titratable acidity (Fig. 3, Table 1 in the supplementary material), the results obtained are in agreement with those observed for the pH parameter. As expected, titratable acidity increases as the fermentation time increases. The increase was more pronounced when using a higher percentage of beans in the formulations containing raw or boiled yam. When raw yams were used (Fig. 3a), the estimated titratable acidity variations were equivalent to 2.45 and 4.55 mL of 1 mol L⁻¹ NaOH per 100 g in the formulations without and with 50.0% bean extract, respectively. Titratable acidity variations of 3.03 and 3.70 mL of 1 mol L⁻¹ NaOH per 100 g were obtained in formulations made with cooked yam without and with 50.0% bean extract, respectively (Fig. 3b). It is important to note that there was a similar behavior to that observed for pH, when using raw or cooked yams. The variation in titratable acidity was more pronounced when the formulation was made with raw yam and enriched with 50.0% bean extract. A titratable acidity increase of 85.7% was obtained when the raw yam was used. Still, the increase in the titratable acidity variation was equivalent to 22.1% when the formulation contained cooked yam.

The cooking of food changes its nutritional value, mainly affecting the content of vitamins and other antioxidant substances. Due to the expected reduction of these compounds in cooked foods, it is possible that cooking of the yam compromised the content of these substances, and consequently negatively affected the pH decrease and the increase in titratable acidity of formulations made with cooked yam. This effect on fermentation of the substrate with cooked yams does not totally impair the fermentation process. Furthermore, cooking the yam is necessary to deactivate various antinutritional factors such as calcium oxalate raphides, which when ingested frequently can cause severe gastrointestinal problems (Silva 2011).

Beans are a good source of protein (18.0–25.0%) and carbohydrates (68.0–76.0%), with starch being the main carbohydrate source (Pires et al. 2005). Yams are a low protein source (2.19–3.0%) but a good source of carbohydrates (Filho et al. 1997). The higher protein content of

Table 1 Chemical composition and color of ingredients and different extracts containing raw yams inoculated or not with water kefir grains, before or after the fermentation

Products	Moisture (%)	Proteins (% d.b.)	Lipids (% d.b.)	Ash (% d.b.)	Carbohydrates (% d.b.)	L	C	h°
Ingredients								
Yams*	73.3	7.87	0.75	4.49	86.89	–	–	–
White beans*	3.90	22.06	52.44	3.02	22.48	–	–	–
Sesame*	12.00	26.55	0.97	3.99	68.49	–	–	–
Extracts								
RY + SE	92.64 ± 0.08a	16.88 ± 2.32cd	38.57 ± 0.21a	4.38 ± 0.14a	40.16 ± 2.48d	74.56 ± 0.07a	4.73 ± 0.04b	97.28 ± 0.22b
WB	89.72 ± 1.35bc	29.14 ± 4.05a	1.55 ± 0.24f	3.78 ± 0.09abc	65.52 ± 3.96b	46.86 ± 0.19f	2.28 ± 0.03c	164.60 ± 4.23a
ⁿ (100.0% RY + SE) + BS	88.70 ± 0.12cd	11.68 ± 0.60d	9.97 ± 0.36d	3.10 ± 0.13bcd	75.24 ± 0.83a	66.93 ± 0.02bc	14.23 ± 0.03a	83.72 ± 0.08c
ⁿ (75.0% RY + SE) + (25.0% WB) + BS	88.01 ± 0.12d	16.81 ± 0.60cd	7.35 ± 0.21e	2.94 ± 0.29d	72.90 ± 0.20a	62.37 ± 0.72d	14.66 ± 0.34a	83.78 ± 0.37c
ⁿ (50.0% RY + SE) + (50.0% WB) + BS	87.44 ± 0.10d	19.12 ± 0.45bc	5.98 ± 0.07e	3.03 ± 0.03cd	71.86 ± 0.48a	58.83 ± 0.26e	14.72 ± 0.13a	83.93 ± 0.09c
^f (100.0% RY + SE) + BS	89.74 ± 0.06bc	16.37 ± 0.86cd	26.96 ± 0.68b	3.65 ± 0.24abcd	53.00 ± 0.93c	65.70 ± 1.13c	14.44 ± 0.30a	83.86 ± 0.12c
^f (75.0% RY + SE) + (25.0% WB) + BS	90.63 ± 0.01b	22.57 ± 1.56b	21.23 ± 1.50c	3.86 ± 0.54ab	52.33 ± 2.60c	67.81 ± 0.37b	14.12 ± 0.06a	83.13 ± 0.26c
^f (50.0% RY + SE) + (50.0% WB) + BS	90.01 ± 0.05bc	23.80 ± 2.22ab	11.39 ± 0.60d	3.52 ± 0.35bcd	61.28 ± 2.41b	62.80 ± 0.70d	14.75 ± 0.54a	83.77 ± 0.19c

*TACO (2011)

RY raw yams, SE sesame, WB white beans, BS brown sugar, d.b. dry basis, L luminosity, C color saturation or chroma, h° Hue angle, ⁿ non fermented extract, ^f fermented extract

Means followed by the same letter in the columns did not differ statistically at 5% probability level Tukey test

Fig. 3 Regression curves of titratable acidity of extracts containing raw yams (**a**) and cooked yams (**b**), sesame and beans inoculated with water kefir grains for 24 h. *RY* raw yam, *SE* sesame, *WB* white beans, *CY* cooked yam

the bean was responsible for the greater decrease in pH and increase of titratable acidity in the formulations enriched with this product. Kefir grains are formed by a complex microbial community consisting of lactic, acetic and yeast bacteria (Gao and Li 2016). Due to this large diversity of microorganisms it is possible that proteolytic bacteria are present, where their growth is favored by the inclusion of beans as a source of protein.

The vegetable drinks developed in this study have an advantage over vegetable juice beverages, such as carrots, fennel and melon, previously described in literature. For example, melon juice has an initial pH of about 6.4. After 48 h of fermentation, the melon beverage drops to pH 4.4 (Corona et al. 2016). In the present study it was observed that formulations containing raw or boiled yams with sesame enriched with 50% bean extract reached a pH of approximately 4.3 in only 24 h. A reduced fermentation time to reach a pH of about 4.4 to 4.6 or lower is desirable, since the chance in development of pathogenic

microorganisms will be much lower, and consequently will not negatively influence the sensory quality of the beverage.

It is emphasized that the pH and titratable acidity determination are considered indirect measures of microbial growth. Hence, we can affirm that the vegetable drinks enriched with beans were able to stimulate greater microbial growth of certain microbial populations during the fermentation process. However, it cannot be confirmed which microbial populations in the kefir grains showed favored growth based on the data obtained so far.

Chemical composition

The chemical composition of the different vegetable drinks prepared with raw yams inoculated and not inoculated with kefir grains, before and after the fermentation process, were evaluated according to Table 1. It was observed that the vegetable beverages with beans and brown sugar added presented a significant reduction ($p < 0.05$) in the lipid percentage. After the fermentation process, it was verified that the formulations with beans continued to present a lower percentage of lipids. The beverage enriched with 50.0% beans presented a significant reduction of lipids ($p < 0.05$) in relation to beverages with 25.0% beans and those without addition of beans. In addition to this desirable nutritional characteristic, the percentage of proteins is significantly higher in the drink with 50.0% beans ($p < 0.05$). It should be noted that before the fermentation process, the carbohydrate content of the beverages was greater than 70.0%.

It was verified that at the end of the fermentation process all beverages presented a reduction in the percentage of carbohydrates. This reduction of carbohydrates can be justified by the presence of lactic bacteria that have homofermentative or heterofermentative metabolism, capable of consuming carbohydrates and generating different types of organic acids and other compounds as metabolic end products. Besides the lactic bacteria, the microbial community present in the kefir grains is enriched with yeasts, which also have the capacity to ferment carbohydrates.

Although the kefir beverage enriched with 50.0% bean extract was that which presented the highest percentage of carbohydrates after fermentation, it was also that which provided the greatest decrease in pH and increase of titratable acidity after 24 h of fermentation. The marked decrease in pH and increase in titratable acidity may also be related to the release of amino acids and fatty acids by proteolytic and lipolytic bacteria, respectively. In this case, lipolytic bacteria were probably those that contributed the most to the decrease in pH. Contrarily, the lowest

Table 2 Chemical composition and color of different extracts containing cooked yams inoculated or not with water kefir grains, before or after the fermentation

Extracts	Moisture (%)	Proteins (% d.b.)	Lipids (% d.b.)	Ash (% d.b.)	Carbohydrates (% d.b.)	L	C	h°
CY + SE	92.37 ± 0.10a	23.00 ± 0.30b	39.53 ± 0.26a	3.45 ± 0.07a	34.04 ± 0.30d	65.90 ± 0.03d	12.24 ± 0.02g	96.59 ± 0.08a
WB	87.11 ± 0.70d	25.93 ± 0.30a	2.90 ± 0.31f	3.10 ± 0.10b	68.07 ± 0.37a	80.00 ± 0.09a	7.21 ± 0.01h	95.71 ± 0.03b
ⁿ (100.0% CY + SE) + BS	90.01 ± 0.16b	15.65 ± 1.35cd	25.44 ± 0.31b	2.37 ± 0.06cd	56.55 ± 1.61c	68.21 ± 0.07b	16.96 ± 0.02f	80.92 ± 0.04c
ⁿ (75.0% CY + SE) + (25.0% WB) + BS	88.88 ± 0.19bc	16.23 ± 0.24cd	17.47 ± 1.24d	2.39 ± 0.18cd	63.90 ± 0.96b	65.38 ± 0.01e	17.66 ± 0.03e	80.40 ± 0.03d
ⁿ (50.0% CY + SE) + (50.0% WB) + BS	85.28 ± 0.32ef	16.13 ± 0.91cd	11.52 ± 0.85e	2.25 ± 0.13d	70.10 ± 1.45a	62.09 ± 0.02h	19.29 ± 0.01c	79.51 ± 0.03e
^f (100.0% CY + SE) + BS	87.75 ± 0.65cd	15.53 ± 1.06d	20.51 ± 1.16c	2.45 ± 0.06cd	61.51 ± 0.30b	66.84 ± 0.01c	18.09 ± 0.02d	79.34 ± 0.01f
^f (75.0% CY + SE) + (25.0% WB) + BS	86.58 ± 0.19de	15.98 ± 0.34cd	16.95 ± 0.86d	2.44 ± 0.14cd	64.64 ± 1.05b	65.00 ± 0.01f	19.45 ± 0.01b	78.59 ± 0.02g
^f (50.0% CY + SE) + (50.0% WB) + BS	84.75 ± 1.09f	17.71 ± 0.46c	16.36 ± 1.50d	2.59 ± 0.08c	63.35 ± 1.97b	63.63 ± 0.02g	21.43 ± 0.01a	78.17 ± 0.02h

CY cooked yams, SE sesame, WB white beans, BS brown sugar, d.b. dry basis, L luminosity, C color saturation or chroma, h° - Hue angle, ⁿ non fermented extract, ^f fermented extract

percentage of lipids was observed in the beverage with 50.0% bean extract.

Table 2 presents the chemical composition of different vegetable drinks prepared with boiled yam and inoculated or not inoculated with kefir grains, before and after the fermentation process. It was observed that boiling of the yam resulted in higher percentages of carbohydrates after fermentation (Table 2) than in the formulations made with raw yam (Table 1). After fermentation, it was expected that the carbohydrate content decreased significantly, as was observed in the formulations made with raw yams. In the formulation elaborated with cooked yam and sesame enriched with 25.0% bean extract, no significant difference ($p > 0.05$) was observed regarding the carbohydrate content before and after fermentation. In the other formulations a significant difference ($p < 0.05$) was observed after fermentation. However, when considering the microbial metabolism, carbohydrate consumption in beverages made with cooked yam was lower than that observed in those with raw yams. It was observed that formulations made with cooked yam showed little apparent variation in protein content. However, when crude yams were used a significant increase ($p < 0.05$) in protein and lipid content after fermentation was observed (Table 1). That increase can be justified by the expressive decrease in carbohydrate content when raw yams were used. In the formulation containing boiled yam and without addition of bean extract, a significant increase of the carbohydrate content was observed in association with a decrease in lipid content.

It is noteworthy that the low carbohydrate consumption by kefir grains in the formulation with boiled yams can be justified by the reduced pH decrease and lower increase in titratable acidity observed in formulations made with raw yams. It is possible that boiling reduced some yam nutrients, which are essential for some microorganisms in kefir grains, thus affecting fermentation and consequently reducing carbohydrate consumption.

Color characteristics

The mean values related to coloration of the different vegetable drink formulations made with raw yams and sesame and enriched or not with bean extract are presented in Tables 1. When the luminosity (L) of the formulations containing raw yam was evaluated, a significant increase ($p < 0.05$) after the fermentation process was observed. According to AMSA (2012), the luminosity can be used to evaluate the extent of product lightening (increase of L*) or darkening (decrease of L*). Therefore, the fermentative process made the beverages lighter. Several factors may be responsible for the significant alteration of luminosity of the fermented formulations. The consumption of brown sugar and the synthesis of several metabolites during

fermentation carried out by the microbial community of the kefir grains may have contributed to the increase of luminosity. The other parameters associated with color C (color saturation) and h° (hue angle) did not show a significant difference ($p > 0.05$) due to the fermentation process.

With regards to color, in drinks made with boiled yams and sesame and enriched or not with bean extract (Table 2), a significant variation in luminosity (L) was observed due to the fermentation process. However, the increase in luminosity was less intense than that observed in beverages made from raw yams. It should be noted that when cooking foods containing starch, such as yams, gelatinization of starch occurs as well as the Maillard reaction. In addition, unlike the beverages containing raw yams, there was a significant variation of the color variables C and h° . The fermentation process caused an increase in C values and reduction in h° values. According to Jacomino et al. (2003), the values are close to zero for neutral colors (gray) and around 60 for intense or vivid colors. In this sense, beverages containing boiled yam showed more intense colors after fermentation, possibly due to the consumption of brown sugar or synthesis of metabolites during the fermentation process.

Conclusion

Fermentation of extracts containing yams, sesame and beans by water kefir grains proved to be suitable for the production of fermented vegetable drinks. Bean supplementation was important to accelerate the fermentation process. Although cooking interferes with the nutritional value of the yam, the reduction of nutrients in the cooked yam did not compromise the pH decrease and the titratable acidity increase during the fermentation process. Thus, the formulation elaborated with yams and sesame enriched with 50% beans was not only the best substrate for obtaining the kefir beverage, but also a protein-rich drink. Development of these kefir vegetable drinks is intended to address the shortage of non-dairy probiotics for the population of vegans or people with allergies to dairy products. It is also an excellent source of vegetable-based protein.

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