A Comparative Analysis of Nutritional and Protein Composition in Vegan and Non-Vegan Milk Products

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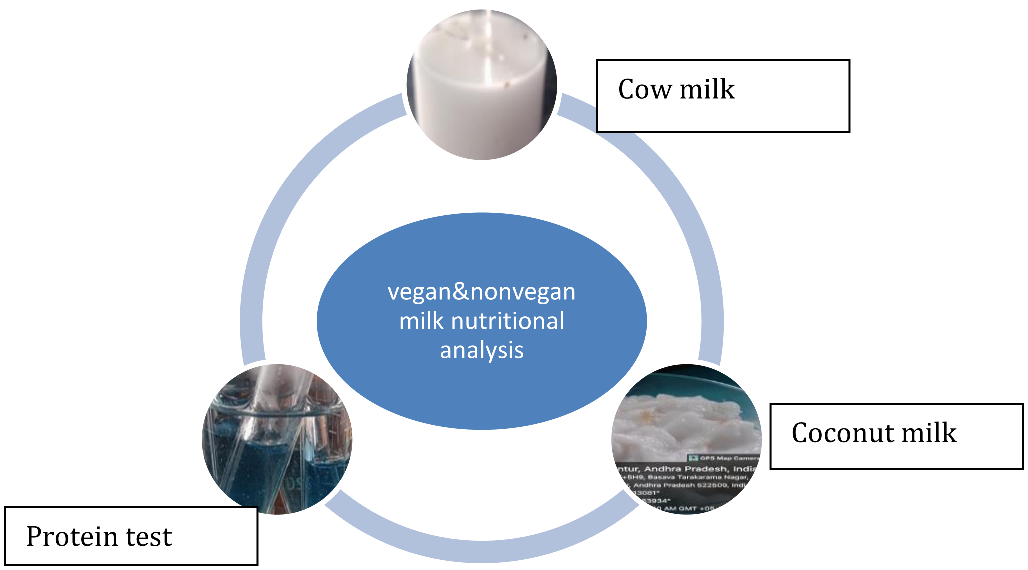
ABSTRACT:

The present study conducts a comparative analysis of the protein and nutritional composition of milk products derived from vegan and non-vegan sources. Milk is recognised for its high protein content and nutritional value. The adoption of plant-based milk by individuals adhering to a vegan lifestyle has become a global phenomenon due to its perceived advantageous effects on health. Plant-based milk foods account for 18% of the total milk sales. The process of extracting milk solids is necessary for the production of milk powders derived from plant sources. The aforementioned product is characterised by its compactness, portability, and extended shelf life in comparison to pasteurised liquid milk. There are multiple factors contributing to this expansion. Lactose intolerance is a crucial factor. The aforementioned dairy substitutes are devoid of lactose and exhibit reduced levels of cholesterol and fat. Individuals with lactose intolerance opt for non-dairy substitutes due to their superior digestibility. The trend of incorporating plant-based ingredients into beverages has been further propelled by the emergence of novel drinks derived from nuts, grains, and seeds, in addition to soy. The nutritional content of a food item is determined by its plant origin and fortification.The present study undertakes a comparative analysis of the nutritional and protein levels of milk products that are vegan and non-vegan in nature. Milk serves as a significant source of protein and essential nutrients. Plant-based milk has been widely adopted as a substitute by vegans globally, owing to its various health benefits. Bovine species, comprising of cattle, buffaloes, as well as camelids, alongside goats and sheep, are the primary sources of milk production worldwide. The consumption of milk holds significance. The constituents that are present in the highest quantities are water, lipids, protein, and lactose. The minor constituents of a substance comprise of various elements such as minerals, enzymes, vitamins, and dissolved gases.

Key words: Milk, proteins, nutrition, lactose, vegan milk and non-vegan milk.

GRAPHICAL ABSTRACT:



INTRODUCTION:

Milk is a healthy, diverse diet that comprises proteins, lipids, and vitamins (Finete et al., 2013). (Clare and Swaisgood (2000)) Proteins are growth factors, hormones, enzymes, antibodies, and immunological stimulants. Proteins are the most vital organic substances. Their biochemical and physiological role in life is known.Mammary glands provide healthy milk for babies. This page discusses cow's milk. Cow's milk produces cheese, cream, butter, and yoghurt, among other foods. Modern diets include dairy or milk products. This cow's milk article covers everything. Protein, a complex component in all living things. Proteins are vital to life's chemical activities. Calcium is primarily linked with healthy bones and teeth, but it also helps muscles contract, clots blood, and regulates heart rhythms and neuron activities. The body stores 99% of its calcium in bones and 1% in blood, muscle, and other tissues..

VEGAN MILK

Cow milk contains all macro and micronutrients, including fat, carbs, proteins, calcium, and vitamins A, B2, and B12 (Haas et al., 2019; Vanga& Raghavan, 2018). Cow milk protein allergy, lactose intolerance, calorie content, and hypercholesterolemia are driving milk substitutes. Vegan and vegetarian diets are also driving vegan milk alternative development (Sethi et al., 2016). Due to lifestyle changes and an increase in autoimmune diseases, fibromyalgia, arthritis, irritable bowel syndrome, and other health issues in people aged 30-50, nutritionists have recommended vegan milks.

Nutritionists and experts recommend vegan milks. Many coffeehouses provide vegan milk as an alternative to cow's milk due to customer behaviour. Vegan milk's nutritional content depends on raw material quality and manufacturing technique (Rincon et al., 2020). Dietary fibres, unsaturated fatty acids, minerals, vitamins, and antioxidants in plant sources have made them functional foods and nutraceuticals (Tangyu et al., 2019). They lack calcium and proteins, making them less nutritious than cow's milk. Polyphenols and trypsin inhibitors bind enzymes and reduce protein digestion. Heat may breakdown or inactivate trypsin inhibitors, although also reduces nutritional value, notably amino acids (OutiElinaMäkinen et al. 2016). Phytate and oxalate, other anti-nutritional chemicals, reduce vitamin and mineral bioavailability. Heat degrades oxalate but not phytate. Fermentation or phytase enzyme may reduce vegan milk phytate (OutiElinaMäkinen et al. 2016).

NON-VEGAN MILK : Western diets consume more non-vegan foods, which provide 17% of calories and 35% of protein [Council for Agricultural Science and Technology et al., 1999]. Since cattle contributes for 18% of global greenhouse gas (GHG) emissions, this high consumption of non-vegan food items is a serious sustainability issue [H. Steinfeld et al., 2006]. The livestock sector's overall GHG emissions and land usage are mostly due to milk production [Food and Agriculture Organisation Greenhouse Gas Emissions from the Dairy Sector A Life Cycle Assessment (2010)].
Therefore, many academic and food business projects attempt to replace dairy with vegan goods.[RabobankDare Not to Dairy(2018)].Dairy products include high-quality protein and a range of micronutrients, making them nutritionally superior to vegan alternatives [M. Springmann Lancet Planet Health,et al.,2 (2018), pp. e451-e461].Vegan proteins' solubility, gelling, and emulsifying qualities are sometimes inferior to milk proteins [S. Qamar, Y.J. Manrique, H. Parekh, J.R. FalconerNuts (2019)].Due to the challenges of replacing milk proteins with plant-based proteins, synthesising nature-identical milk proteins may be a solution. Several firms are investigating dairy product manufacture using recombinant milk protein synthesis. This study will cover the features of important milk proteins, the present status of recombinant milk protein synthesis, and upcoming research difficulties before animal-free dairy products may be made.

MATERIALS AND METHODS: The methods of sampling and analysis mentioned in the manuals as specified by the Food Safety and Standards Authority of India from time to time shall be applicable.

A) **DETERMINATION OF PROTEIN** : ***BIURET REAGENT*** – take 500 ml distilled water 0.4 gm of NAOH (Sodium hydroxide) mixed at glass rod, add 1.5gm of CUSO4 ,4.5gm of KNaC4H4O6.4H2O , 2.5 of KI . ***Protein strand:*** 250mg of C6H8N2O4 (Egg Albumin) and add 50 ml of distilled water. Checking the results with the help of cuvettes.

B) **DETERMINATION OF KJELDAHL METHOD:**a sample is mineralized by boiling (with concentrated H2SO4); N is then transformed from its different functional group forms to NH3; N is then bound in the form of (NH4)2SO4; and N is released from the (NH4)2SO4 by alkalization and titrated. [IS USED: milk; Refer to COSMT (2014a, b)]

C) **DETERMINATION OF CALCIUM**:In a 10 mL volumetric bottle, a certain amount of Ca^{2+} working solution (conditional experiment, 4.0 μg Ca^{2+}) was added, and 2.5 mL of 0.050% (W/V) dibromo-*p*-methylsulfonazo solution and 1.5 mL of 4 mol/L hydrochloric acid were added. Water was added to the mark. It was allowed to stand for 30 min. Take test tubes add 3ml sample and add calcium reagent, At the wavelength of 624 nm, the absorbance of the chromogenic solution was determined with 1 cm cells by using the corresponding reagent blank as the reference.

D) **DETERMINATION OF VITAMINS:**Weigh, to the closest 0.01 g, around 30 g of solid milk and dairy products into a 500 mL flask, dissolve the sample in 180 g of warm water (40°C to 45°C), and mix until homogenous. Shaken milk may be weighed. To resist 100°C, reconstituted milk was carefully weighed into a 50 mL centrifuge tube. 100 µL of [13C7]-vitamin B12 working standard solution (50 ng/mL), 25 mL of sodium acetate solution, 10 mg of Takadiastase, and 1 mL of potassium cyanide (1%) were added, agitated, and incubated at 37°C for 30 min in thermostatic oscillator. The hydrolysates spent 30 minutes in a 100°C water bath. The solution was shaken and centrifuged for 10 min at 8000 r/min after cooling to room temperature. After filtering, the supernatant was purified. A glass adapter loaded all filtrate onto an immunoaffinity column. The column was cleaned and dried with 10 mL of water and air. Complete antibody denaturation eluted vitamin B12 three times into a 10 mL glass tube with 1 mL of methanol. Before HPLC analysis, the eluate was dried at 60°C under slow nitrogen gas flow, reconstituted in 1 mL of mobile phase, and filtered through a 0.22 μm membrane filter.

E) DETERMINATION OF LACTOSE (BENEDICTS METHOD): Pour 5 ml of milk sample into flask and add 2.5 ml of 10% sodium tungestate drop by drop while mixing. Continuously combine 5 ml of 0.5 N sulphuric acid. Finally, add 50 ml distilled water. Filter after 10 minutes. Burette the filtrate. 25 ml of benedicts reagent, 30 ml of dis.water, and 2 gramme of anhydrous sodium carbonate in another beaker. Mix thoroughly and heat until the solution is clear. While boiling, titrate quickly by 2 ml until the first shade of reduction is reached. Titrate until blue disappears. Reddish brown replaces blue. Record the titration filtrate volume (ml).

CALCULATION

$$\text{LACTOSE \%} = \frac{0.0678}{R} \times 10 \times 100$$

F) DETERMINATION OF FAT : Take a soxhlet bulb fill 50ml distilled water and fixed the extractor place the thimble into the extractor and place the milk powder into the thimble& add 50ml distilled water. fix the condenser top of the extractor, checking it water passing in 1 hour (Inlet and outlet). Fix the boiling temperature 35^{0}C.Overall, the Soxhlet technique of extraction is a powerful tool that can be used to produce high-quality extracts from a variety of milks.

G) DETERMINATION OF ASH : Weigh a clean, dry crucible. To determine sample weight, weigh 2 g of sample. Carefully set weighted crucible on electric hob. Half-open the crucible. Smoke will burn the sample. Heat crucible to 600oC in muffle furnace. 2 hours. This temperature burns all biological substance, leaving minerals. Remove the crucible from the furnace and cool it in a dessicator.

Calculation

Ash content (%) = (Z – X /Y – X) x 100

Weight of empty crucible - X g

Weight of crucible + sample - Y g

After complete ashing, Weight of crucible + ash - Z g

What is obtained after complete combustion of a sample is total ash. (Reference: AOAC Official Method 942.05.)

H] DETERMINATION OF CARBOHYDRATES METHOD: Dissolve 1.25gm of glucose AR in water in a 250 mL standard flask to make a standard solution. Fill the volume. Shake 20 mL of Fehling's A and B in a dry conical flask. Pipette 20 mL of this newly mixed Fehling's solution into a clean conical flask and dilute with 20 mL water. Heat wire gauze with 70° solution. Run the burette-prepared standard glucose solution gently into the boiling Fehling's solution until the blue colour disappears. Keep this temperature while adding glucose solution. Repeat the foregoing titration by slowly adding glucose solution to the boiling Fehling's solution until the end point is achieved. To more reliably identify the end point, add 5-6 drops of methylene-blue indicator to Fehling's solution and then glucose solution drop by drop. If methylene-blue is the indicator, Fehling's solution should not boil for longer than 2-3 minutes. The blue colour likewise disappears in the end.

RESULTS AND DISCUSSION

Fig:1.Brick red colur confirms glucose conformation

MACRONUTRIENT Characteristics of the vegan and non-vegan milks: The macronutrient study of protein, fat, and carbohydrate content comprised samples of 3 bovine whole milks (3.5% fat) and 4 plant-based beverages. The following carbs were tested in all plant-based milks and non-vegan milk: sucrose, fructose, glucose, lactose, starch, and fibre. The plant-based beverages' total carbohydrate levels varied from 13.2 g/Kg-1 in almond milk to 48.2 g/Kg-1 in rice milk. Almond milk had a high total protein content of 10.2 g/Kg-1 whereas rice milk had a low level. Almond milk was found to have the greatest fat content (25.6g/Kg-1).

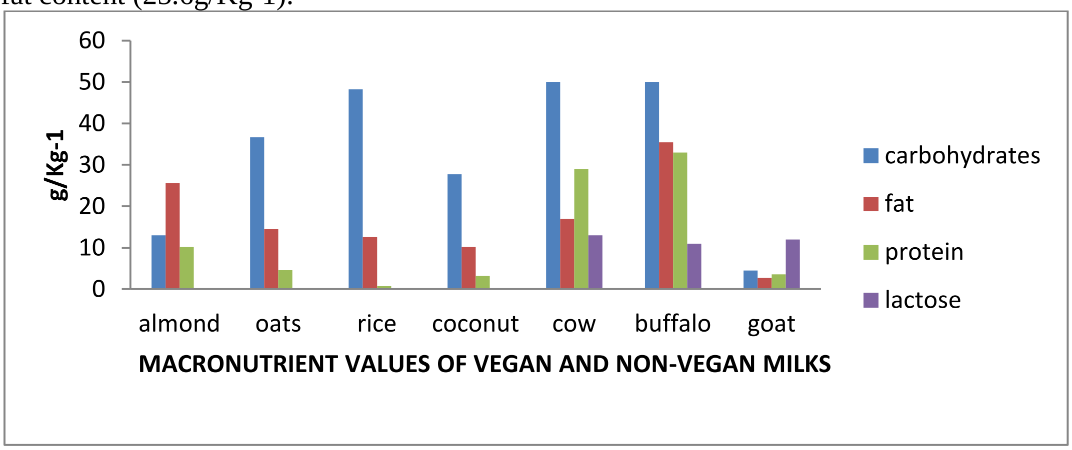


Fig 2: Macronutrient analysis of vegan and non-vegan milks.

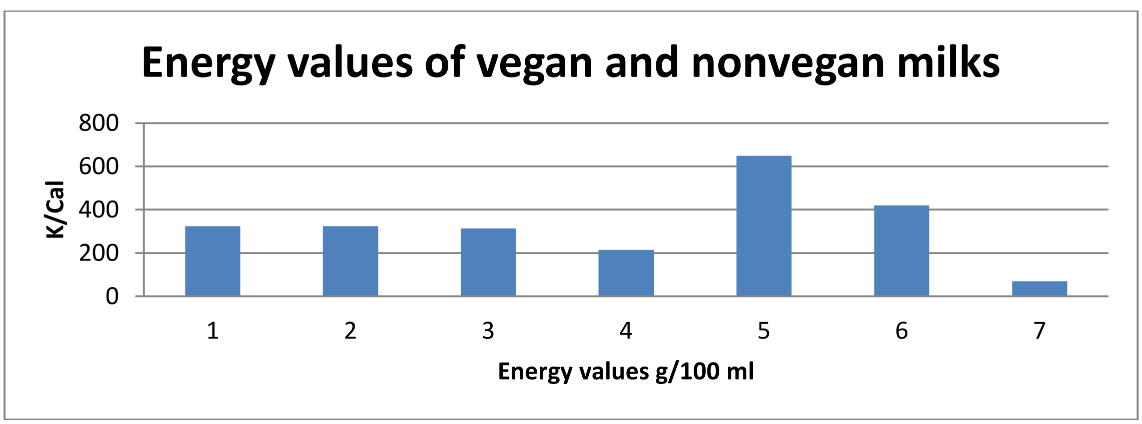


Fig 3: energy values of vegan and non-vegan milks.

MICRONUTRIENT Characteristics of the vegan and non-vegan milks:

The study incorporated a set of 4 plant-based beverages and 3 samples of bovine whole milk with a fat content of 3.5% for the purpose of conducting a macro nutrient analysis. The results of this analysis, which included the representation of vitamins and calcium, are depicted in Figure 4. Vitamins A, B12, C, and D were quantified in both plant-based and non-vegan milk varieties. The Vitamin A levels in non-vegan milk varied between 90 µg/100 ml in goat milk and 46 µg/100 ml in cow milk. Almond milk and goat milk were found to have high Vitamin D content, measuring at 6 µg/100 ml and 16 µg/100 ml, respectively.

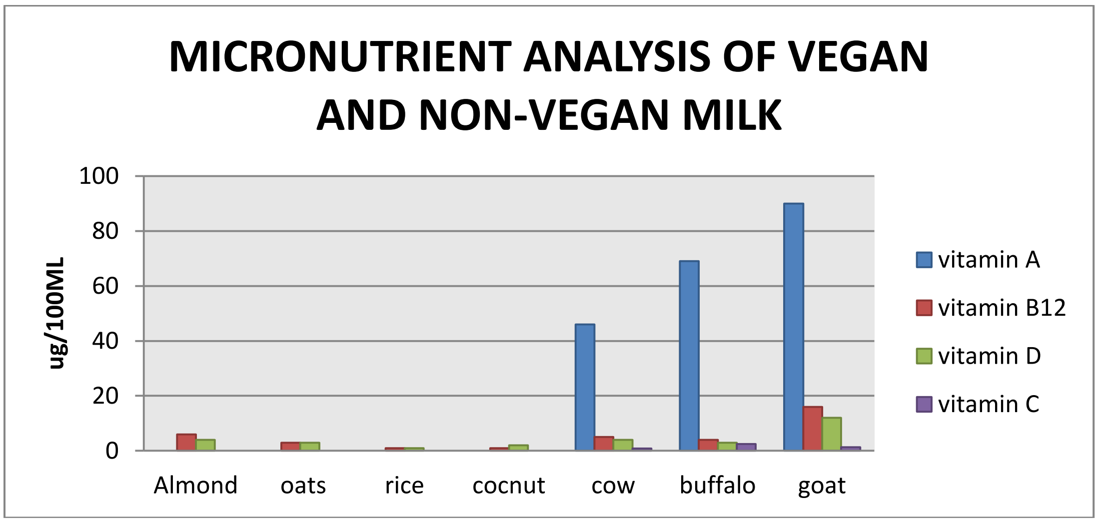


Fig 4: micronutrient analysis of vegan and non-vegan milks.

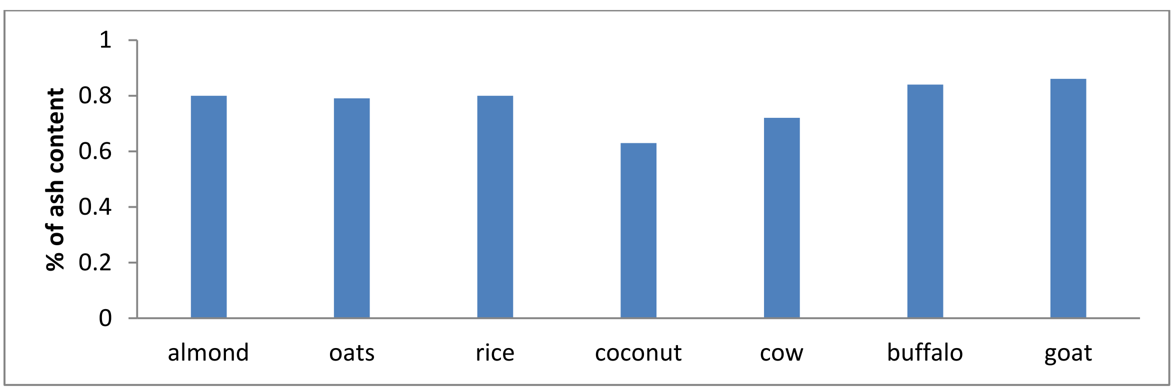


Fig 5: Ash content (%) of vegan and non-vegan milks.

CONCLUSION : A 200 mL serving of cow's milk provides more than 10% of the recommended daily allowance (RDA) for biotin, pantothenic acid, vitamin B2, phosphorus, calcium, and iodine, as indicated in Supplementary Table 7. Additionally, it has the potential to contribute a substantial amount of the recommended daily intake of protein that is of superior nutritional value for human consumption. Milk offers an additional benefit in terms of its relatively greater stability in nutrient composition, with the exception of iodine levels which may fluctuate based on seasonal and dietary factors (89). Bovine milk serves as a noteworthy source of fatty acids, exhibiting a noteworthy ratio of omega 6 to omega 3. However, it is important to note that saturated fatty acids remain dominant and the consumption of trans fatty acids is still prevalent. Plant-based beverages exhibit a clear advantage owing to their elevated proportions of monounsaturated and polyunsaturated fatty acids, with the exception of coconut-based beverages. Plant-based beverages, specifically those derived from soy, as well as products like infant formulas, are recommended for individuals who suffer from cow's milk protein allergies and intolerances, hereditary lactase deficiencies, and galactosemia. The presence of antinutrients and insufficient levels of certain essential amino acids (41) often diminishes the digestibility and nutritional efficacy of plant-based proteins. While lactose intolerance is commonly associated with cow's milk lactose, it is important to note that soy milk also contains raffinose and stachyose, which have been known to cause digestive discomfort. The presence of phytic acid in plant-based products warrants consideration due to its potential to impede the bioavailability of crucial minerals, even in instances where said minerals are present within the products. Hence, it is imperative to conduct regular assessments of the anti-nutrient composition of plant-based commodities and disseminate the findings to the general public. Plant-based beverages have limited capacity to offer substantial quantities of micronutrients in the absence of fortification, in contrast to cow's milk, which is a more abundant source of nutrients such as vitamin B12 and iodine (90, 91). Of the plant-based beverages that were tested, soy-based beverages exhibit nutritional characteristics that are most similar to those of milk in terms of their contribution to recommended daily allowances (RDAs). However, it is important to exercise caution before completely replacing milk with soy-based drinks. Furthermore, it is noteworthy to state that further scientific evidence is needed regarding the enduring impact of consistent consumption of soy phytoestrogens (namely idaidzein, genistein, glycitein) on endocrine functions and the reproductive system in the future, particularly in children (41). In the future, it is imperative to give equal consideration and communication to the nutritional quality of plant-based drinks, which should be supported by scientifically proven nutrient and micronutrient bioavailability, alongside sustainability goals (92). Feasible strategies for addressing nutritional deficiencies in certain plant-based beverages may involve selecting a variety of plant-based substitutes as a component of a well-rounded dietary regimen to guarantee sufficient fulfilment of essential nutrients and micronutrients. Finally, there are genuine opportunities for innovation in moving away from complex and ultraprocessing food manufacturing methods, which are typically necessary in the production of plant-based products, towards simpler and/or natural processes like microbial fermentation that can lessen the antinutritional qualities of foods and ingredients, enhance protein digestibility, and produce more micronutrients and phytonutrients.

REFERENCE:

1. Ash: AOAC 942.05, AOAC 945.46, AOAC 923.03
2. Calcium: Varian Flame Atomic Absorption Spectrophotometry Analytical Methods/ and Spectra ASoftware 220 FSAOAC 985.35
3. CLARE, D. A. and SWAISGOOD, H. E. 2000. Bioactive milk peptides: a prospectus. J. Dairy Sci., 83(6): 1187–1195.CNI. 1973. Methods for testing of milk and milk products [in Czech: Metodyzkoušenímléka a tekutýchmléčnýchvýrobků]. CSN 57 0530:1973. Prague: Czech Normalization Institute.
4. COSMT. 2014a. Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein [in Czech: Mléko-Stanoveníobsahudusíku-Část 1: MetodadleKjeldahla]. CSN EN ISO 8968-1:2014 (57 0528). Prague: Czech O ce for Standards, Metrology and Testing.

5. COSMT. 2014b. Milk – Determination of nitrogen content - Part 2: Block-digestion method (Macro method) [in Czech: Mléko – Stanoveníobsahudusíku – Část 2: Metoda s blokovoumineralizací (Makrometoda)]. CSN EN ISO 8968-2:2014 (57 0528). Prague: Czech O ce for Standards, Metrology and Testing

6. Council for Agricultural Science and Technology Animal Agriculture and Global Human Food Supply (1999)
7. Fat: Automated Soxhlet Method/Tecator and AOAC 936.15
8. FINETE, V. DE L., GOUVÊA, M. M., MARQUES, F.F. et al. 2013. Is it possible to screen for milk or whey Protein adulteration with melamine, urea and ammonium sulphate, combining Kjeldahl and classical Spectrophotometric methods? Food Chem., 141(4): 3649–3655
9. Food and Agriculture Organization Greenhouse Gas Emissions from the Dairy Sector A Life Cycle Assessment (2010)
10. Haas R, Schnepps A, Pichler A, Meixner O. (2019). Cow milk versus plant-based milk substitutes: A comparison of product image and motivational structure of consumption. Sustainability, 11(18), 5046.
11. H. Steinfeld, P. Gerber, T. Wassenaar, V. Castel, M. Rosales, C. de HaanLivestock's Long Shadow (2006)
12. J. Poore, T. NemecekReducing food's environmental impacts through producers and consumersScience (80-), 360 (2018), pp. 987-992
13. Mäkinen OE, Wanhalinna V, Zannini E, Arendt EK. Foods for Special Dietary Needs: Non-dairy Plant-based Milk Substitutes and Fermented Dairy-type Products. Crit Rev Food SciNutr. 2016; 56(3):339-49. Doi: 10.1080/10408398.2012.761950. PMID: 25575046.
14. Moisture: Air Oven Method, AOAC 930.15, AOAC 945.03, AOAC 925.23AOAC 985.35
15. M. Springmann, K. Wiebe, D. Mason-D'Croz, T.B. Sulser, M. Rayner, P. Scarborough Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detailLancet Planet Health, 2 (2018), pp. e451-e461
16. Protein: Automated Kjeldahl Method/Tecator andAOAC 948.15
17. RabobankDare Not to Dairy (2018)
18. Rincon L, Botelho RBA, de Alencar ER. (2020). Development of novel plant-based milk based on chickpea and coconut. LWT-Food Science and Technology, 128, 109479.
19. Sethi S, Tyagi SK, Anurag RK. (2016). Plant-based milk alternatives an emerging segment of functional beverages: a review. Journal of Food Science and Technology, 53(9), 3408-3423.
20. S. Qamar, Y.J. Manrique, H. Parekh, J.R. FalconerNuts, cereals, seeds and legumes proteins derived emulsifiers as a source of plant protein beverages: a reviewCrit Rev Food SciNutr, 60 (2019), pp. 2742-2762, 10.1080/10408398.2019.1657062
21. Tangyu M, Muller J, Bolten CJ, Wittmann C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. Applied Microbiology and Biotechnology, 103(23), 9263-9275.
22. Vanga SK, Raghavan V. (2018). How well do vegan alternatives fare nutritionally compared to cowsmilk?. Journal of Food Science and Technology, 55(1), 10-20.

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