Nutritional Composition of Novel Plant-Based Meat Alternatives and Traditional Animal-Based Meats

Article in Food Science & Nutrition - July 2021
DOI: 10.24966/FSN-1076/100109

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Nutritional Composition of Novel Plant-Based Meat Alternatives and Traditional Animal-Based Meats

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Abstract

Plant-Based Meat Alternatives (PBMA) are increasing in popularity and may provide essential nutrients for some populations. Therefore, different formulations of popular PBMAs, such as Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), and Morning Star’s Black Bean Burger (BBB), were assessed in comparison to traditional Animal-Based Meats (ABM), such as 80/20 Ground Pork (GP) for nutrient composition. Sodium content was considerably greater (P<0.05) in all PBMAs than GP, along with total saturated fat content being numerically greater when compared to GP. Vitamin E content of all PBMAs was numerically greater than ABMs. Total Essential Amino Acid (EAA) content was numerically greater in BMB2 than in ABMs, although anabolically important EAA, such as methionine and lysine were substantially greater (P<0.05) in GP. While PBMAs were comparable to ABMs in many nutrients, bioavailability should be further investigated.

Keywords: Animal-based meats; Essential amino acids; Nutrition; Plant-based proteins; Saturated fatty acids

Introduction

Poor nutrition and food insecurity are widespread challenges facing the world, with global estimations suggesting that, every year, over 2 billion people may suffer from a nutrient deficiency, 690 million people may suffer from hunger, 144 million children, under the age of 5, may suffer from stunted growth, and over 650 million people may suffer from obesity worldwide [1-4]. Additionally, an increasing global population, accompanied by changes in urbanization, industrialization, and globalization have compounded the challenges regarding poor nutrition and food insecurity as the global demand for animal proteins is expected to double by 2050 [5-7]. Additionally, plant-based diets, such as veganism, vegetarianism, and flexitarianism are increasing worldwide, which has generated a growing affability to plant-based diets, as well as a demand for the development of novel plant-based meat alternatives [8,9].

Plant-Based Meat Alternatives (PBMA) are products that mimic the taste, appearance, or texture of Animal-Based Meats (ABM) [10]. Novel PBMAs have become popular in recent years, with the market growing at five times the rate of the ABM market in 2019 and is expected to reach a global value of $8.1 billion by the year 2026 [11]. Commercial PBMAs are primarily being developed by companies such as Beyond Meat and Impossible Foods; however, traditionally ABM producing companies, such as Cargill, JBS, Tyson, and others are also developing novel PBMAs [12].

To mimic the elasticity and mouth feel of traditional ABMs, modern PBMAs often utilize isolated proteins obtained from legumes, oils, and cereals, as well as purified fats and oils obtained from coconuts, cocoa fruit, sunflower seeds, and rapeseed [12,13]. While many of these plant-sourced foods can often be a good source of dietary nutrients, anti nutrient factors present in plant tissues, such as oxalates, phytates, tannins, and other antagonists, may inhibit the absorption of nutrients [14,15]. In contrast, ABMs have been long regarded as a good source of readily bio available nutrients; but have also been criticized as a potential risk factor for the development of various human diseases [16-18]. Therefore, a substantial knowledge gap exists about the nutrient profiles of PBMAs and ABMs, and its impact on human health and wellbeing [9].

Materials and Methods

Sample collection and processing

To adhere to the United States Department of Agriculture (USDA), Agricultural Research Service’s (ARS) Food Data Central Database guidelines, original and current formulations of the Beyond Meat Burger (BMB1, BMB2), Impossible Foods Burger (IFB1, IFB2), Morning Star’s Black Bean Burger (BBB), along with the 80/20 Ground Pork (GP) were purchased at food service companies and supermarkets from six randomly selected cities (Seattle, WA; Peyton, CO; Memphis, TN; Newburgh, IN; Houston, TX; and Brooklyn, NY) throughout the United States with ingredients listed in table 1. Approximately 5 lbs. of frozen product, with the same lot number, were purchased and transported under refrigeration (4°C) to Colorado State University (Fort Collins, CO), where they were frozen (-20°C) until further analysis. Six replicates (n=6) of each product (i.e., one repliclicate per city), designated as raw or cooked, were analyzed for nutrient content separately. Nutrient profiles for raw and cooked 80/20 Ground Beef (GB) were retrieved from the USDA-ARS Food Data Center nutrient database and utilized for comparisons in the present
work [19,20]. Finally, Recommended Dietary Allowance (RDA) information was retrieved from the United States Department of Health and Human Services, National Institutes of Health database, and a serving size of 113g (4 oz) was utilized for all RDA comparisons [21].

All products were formed into 113g (4 oz) patties and cooked on a pre-heated, non-stick anodized aluminum skillet to an internal temperature of 71°C. After cooking, the product was placed on a stainless-steel rack to cool for 10min. Both uncooked and cooked patties were chilled at refrigerated temperatures (0 to 4°C) for 12 to 24h prior to homogenization. Samples were then frozen in liquid nitrogen and immediately homogenized for 10s on a low speed (1500rpm) and 30s on a high speed (3500rpm) with a Robot Coupe BLITZER 6V (Robot Coupe USA Inc., Ridgeland, MS) blender, until a uniform powder was obtained. Homogenized samples of raw and cooked products were stored at -80°C for further analysis. Unless otherwise indicated, analyses were performed at the Colorado State University (Fort Collins, CO).

Proximate and fiber analysis

Moisture and ash: Moisture analysis was performed using the Association of Official Agricultural Chemists (AOAC) oven drying method 950.46 [22]. Approximately 1g of samples were weighed into aluminum pans and allowed to dry for 24h at 100°C in a forced air-drying oven. Percent moisture was calculated by determining the percent difference between wet and dry weight. Percent ash was determined using the ashing method described by 923.03 of the AOAC official methods [22]. Approximately 1g of homogenate was placed into a pre-weighed crucible and set in a Thermolyne box furnace at 600°C for 18h. Percent ash was calculated by dividing the ash weight by the original wet sample weight and multiplying by 100 to obtain a percentage. All proximate data in the present work are reported on an as-received basis, as opposed to a dry-matter basis.

Crude fat and crude protein: Total lipid content was extracted using the method described by Folch et al., along with processes described in AOAC official method 983.23 [23,24]. Approximately 1g of sample was homogenized in a 2:1 ratio of chloroform and methanol solution, respectively. Homogenized samples were placed onto an orbital shaker at room temperature for 20min, followed by filtering through ashless filter paper. Four mL of 0.9% NaCl was added to the filtered sample, and the sample was placed in a refrigerator (3±2°C) for 24h. When the filtrate separated into two phases, the lower phase was aspirated and placed into a pre-weighed scintillation vial. The liquid inside the vial was then dried under N2 gas. Following drying, the vial was allowed to air dry under a fume hood for 2h and then placed into a forced air-drying oven to dry for 12h at 100°C. Percent fat was determined by dividing the fat weight by the original wet sample weight and multiplying by 100 to obtain a percentage.

Crude protein content was determined according to the AOAC method 992.15 x utilizing a TruSpec CN Carbon/Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Percent protein was calculated by multiplying the total percentage of nitrogen by a factor of 6.25 [24].

Inductively Coupled Plasma (ICP) mass spectrometry analysis

Minerals (Ca, Mg, K, Na, Fe, Zn, Cu, Mn, P) were analyzed by Eurofins Laboratories (Madison, WI) using the USDA wet-ashing procedure and AOAC official methods 985.33, 984.27, 985.01 and 2011.14 [24,25]. Samples were digested in a microwave or on a hot plate with nitric acid, hydrochloric acid, or hydrogen peroxide. The amount of each element was determined with an ICP mass spectrophotometer (Agilent 8900, Santa Clara, CA) by comparing the emission of the unknown sample against emissions from standard solutions.

Vitamin analysis

Fat-soluble vitamins: Unless otherwise specified, vitamin analyses were performed at Eurofins Laboratories (Madison, WI). Vitamin A content was measured using HPLC methods described by Njeru et al. and Alousila et al. [26,27]. Vitamin D and 25-hydroxy-vitamin D analyses were performed using HPLC with UV detection according to AOAC method 2010.11 [25]. Vitamin E content was measured by Craft Technologies (Wilson, NC) using HPLC with a normal phase column as described by Speek et al. [28]. Ultraviolet detection with external calibration was performed as described by Cort et al. with an internal standard recovery as described by McMurray et al. post-analysis [29,30]. Vitamin K1 analysis was performed using AOAC official method 999.15, including HPLC and fluorescence detection [24].

Water-soluble vitamins: B-vitamins were analyzed according to the AOAC official methods utilized in the analysis of each vitamin was as follows: niacin AOAC 944.13 and 960.46; vitamin B-6 AOAC 961.15; riboflavin AOAC 960.46 and 940.33; thiamin AOAC 942.23, 953.17, and 957.17; pantothenic acid AOAC 945.74, 960.46, and 992.07 [24].
Fatty acid and cholesterol analysis

Total lipids were extracted from 1 g of the homogenized sample using methods from previously developed protocols [23,31]. Saponification and methylation of lipids were accomplished using the method described by Parks and Goins [32]. Individual lipids were separated via gas chromatography using a Hewlett Packard (Avondale, PA) Model 6890 series II gas chromatograph fixed with a series 7683 injector and flame ionization detector and fitted with a 100m x 0.25mm fused silica capillary column (SP-2560 Supelco Inc. Bellefonte, PA).

Cholesterol content was analyzed at Eurofins Laboratories (Madison, WI) using AOAC official method 994.10 [22]. Samples were saponified using ethanolic potassium hydroxide. The unsaponifiable fraction that contained cholesterol and other sterols was extracted with toluene. Toluene was evaporated, and the residue was dissolved into dimethylformamide. Samples were derivatized to form trimethyloxysilylethers, and content was quantitatively determined by gas chromatography using 5 alpha-cholesterol as an internal standard.

Amino acid analysis

Amino acid profile was analyzed by Eurofins Laboratories (Madison, WI). Samples were hydrolyzed in 6 N hydrochloric acid for 24h at approximately 110°C. Phenol was added to the 6 N hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid, as described by Barkholt and Jensen [33]. Tryptophan was hydrolyzed from proteins by heating at approximately 110°C in 4.2 N sodium hydroxide as described by AOAC official method 988.15 [22]. Samples were analyzed by HPLC after pre-injection derivatization as described by Henderson et al. and Henderson and Brooks [34,35]. Primary amino acids were derivatized with o-phthalaldehyde and secondary amino acids were derivatized with fluorenylmethyl chloroformate before injection as described by Schuster [36].

Statistical analysis

Statistical analyses were performed using R software (v.3.6.1), whereby simple means and standard deviations for each nutrient component were obtained [37]. The Anova type III function from the Car package was used to determine statistical differences at an alpha level of 0.05 [38]. The emmeans function with a CLD display from the emmeans package was utilized to identify respective statistically significant differences [39]. Tukey adjusted pair wise comparisons were used for each test. Results for nutrient profiles of BMB1, BMB2, IFB1, IFB2, BBB, and GP were reported as least square means (n=6) with standard deviation and a letter superscript designating statistical difference. Results for GB are reported as means with no standard deviation and no statistical superscript, as data was directly retrieved from USDA-ARS Food Data Central Database as a mean, with no standard deviations [19,20]. Finally, nutrient intakes per serving size were determined by multiplying the nutrient component mean by 113g/serving.

Results and Discussion

Proximate analysis

Results from proximate analysis of raw and cooked samples are reported in tables 2 and 3, respectively. In general, crude protein and crude fat content did not differ (P>0.05) for each PBMA and GP in raw and cooked states; although, GB contained the numerically greatest crude protein and crude fat content after cooking. These findings are important, validating that PBMAs are comparable to ABMs in crude protein and crude fat content.

Mineral analysis

Results from mineral analysis for previous and current formulations of raw and cooked samples are reported in tables 4 and 5, respectively. Calcium and sodium were considerably greater (P<0.05) in PBMAs (BMB1, BMB2, IFB1, IFB2, and BBB) than in GP. One serving of cooked PBMA could supply between 3.0 to 24.4% of the adult (19 to 30 years of age) calcium Recommended Dietary Allowance (RDA) for males and females. The Dietary Guidelines for Americans suggest lowering dietary sodium intake, although one serving of PBMA may supply between 27.8 to 42.7% of the adult sodium RDA, and one serving of GB may supply approximately 22% of the adult RDA for sodium [40,41]. In general iron and zinc content was greater (P<0.05) in most PBMAs than in GP, although zinc content was numerically greatest in GB than in the PBMAs and GP. One serving of cooked PBMA may supply between 28.8 to 83.7 % and 15.3 to 44.7 % of the iron RDA for adult males and females, respectively [40]. Nonetheless, iron found in plant sources is exclusively non-heme iron, which is less bio available than heme iron found in GP and GB [42].

While these minerals are nutritionally essential, the presence of phytates, fibrous plant materials, mineral antagonists, and the incorporation of minerals in the food matrix may inhibit absorption [43-46]. High calcium and magnesium levels have been known to decrease iron and potassium absorption, while high iron concentrations have been known to contribute to lower manganese absorption [47-49]. While it has been demonstrated that calcium doses (similar to those present in IFB1) do not significantly reduce iron absorption, calcium has been known to inhibit iron absorption when minerals were ingested from the same food, which could be the case in PBMAs. Additionally, high sodium and potassium levels may increase urinary mineral losses [48-51].

Vitamin analysis

Results from vitamin analysis for previous and current formulations of raw and cooked samples are reported in tables 6 and 7, respectively. Fat-soluble vitamin A, D, E, and K were found to be below the detection limit (<0.3mcg/g for vitamin A, and <0.001 mcg/g for vitamin D, and K) in all raw and cooked products, with only vitamin K being detected in BMB1, BMB2, and BBB samples at concentrations slightly above the detection limit. Vitamin E content in raw and cooked PBMAs was substantially greater (P<0.05) than in raw and cooked GP. Vitamin E content in GB can be variable depending on the production and processing parameters, although GB was numerically less than the PBMAs and GP in the samples tested for this study [52]. One serving of cooked PBMA may supply between 14.7 to 60.1 % of the RDA for adults (19-30y) [40]. Generally, vitamin E supplementation in foods contributes a significant portion (>50% RDA) to the American diet, but absorption of vitamin E in the human intestine is highly variable and can be impacted by the amount consumed, fat content, food matrix, and the presence of other fat-soluble nutrients [53,54].

For each B-vitamin, IFB1 and IFB2 were statistically greater (P<0.05) than or numerically comparable to GP, excluding niacin (B3) and pantothenic acid (B5), for which GP was greater (P<0.05). One serving of IFB1 or IFB2 would surpass the adult thiamin (B1) RDA
Table 2: Proximate analysis (percent/SD) of two formulations of raw Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component | BMB1 | BMB2 | IFB1 | IFB2 | BBB | GP | GB* |
|-----------|------|------|------|------|-----|----|-----|
| Dry Matter | 32.1±0.8* | 42.9±0.8* | 37.2±0.8* | 42.3±0.4* | 32.5±2.7 | 37.1±1.3 | 38.0 |
| Moisture | 67.9±0.8* | 57.1±0.8* | 62.8±2.8* | 57.7±0.4* | 67.5±2.7 | 62.9±1.3 | 62.0 |
| Ash | 1.5±0.7* | 1.7±0.2* | 1.5±0.5* | 2.5±0.2* | 1.3±0.4* | 1.8±0.4* | 0.84 |
| Crude Fat | 10.8±3.8* | 13.1±2.2* | 12.0±5.0* | 11.7±1.9* | 10.8±3.8* | 10.2±4.7 | 20.0 |
| Crude Protein | 20.3±3.4* | 18.6±0.9* | 22.0±4.1* | 17.2±0.9* | 24.0±6.9 | 22.6±3.2 | 17.0 |

Note: ** means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [19].

Table 3: Proximate analysis (percent/SD) of two formulations of cooked Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component | BMB1 | BMB2 | IFB1 | IFB2 | BBB | GP | GB* |
|-----------|------|------|------|------|-----|----|-----|
| Dry Matter | 36.7±3.5* | 50.3±0.9* | 45.0±0.7* | 47.7±1.0* | 30.7±2.9* | 44.8±2.2* | 44.0 |
| Moisture | 63.2±3.5* | 49.7±0.9* | 55.0±0.7* | 52.3±1.0* | 69.2±2.6* | 55.2±2.2* | 56.0 |
| Ash | 1.8±0.6* | 2.1±0.2 | 1.6±0.2* | 3.0±0.2 | 1.5±0.4* | 1.3±0.4* | 1.0 |
| Crude Fat | 11.9±5.2* | 11.6±3.9* | 9.2±3.1 | 11.2±1.7* | 11.9±4.3* | 11.1±5.9* | 18.0 |
| Crude Protein | 23.3±4.4* | 23.8±1.5* | 20.3±3.6* | 20.2±5.0* | 22.4±4.9* | 21.3±3.0* | 26.0 |

Note: ** means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [20].

Table 4: Mineral composition (ppm/SD) of two formulations of raw Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component | BMB1 | BMB2 | IFB1 | IFB2 | BBB | GP | GB* |
|-----------|------|------|------|------|-----|----|-----|
| Calcium | 213.8±11.6e | 819.8±74.0b | 257.5±6.7c | 1860±46.5a | 881.8±40.5b | 105.8±61.9d | 180 |
| Magnesium | 190.8±7.8c | 350±30.1b | 120.7±7.2d | 714±19.3a | 367.3±9.0b | 178.5±16.3c | 170 |
| Phosphorus | 1888.3±74.4b | 2423.3±160.7a | 1296.7±28.8d | 1840±54.7b | 1263±48.4d | 1596.7±126.6c | 1580 |
| Potassium | 2828.3±188.1b | 2411.7±247.9c | 3096.7±89.4b | 5760±197.9a | 2930±129.6b | 3176.7±702.7b | 2700 |
| Sodium | 3328.3±205.5b | 3230.0±275.3b | 4935±167.0a | 3688.3±203.9b | 3273.3±191.0b | 995.5±1281.4c | 660 |
| Copper | 3.4±0.4a | 2.1±0.3b | 3.8±0.4a | 2.7±0.5b | 2.4±0.16b | 0.7±0.1c | 0.61 |
| Iron | 43.4±2.9a | 36.6±2.6b | 22.3±0.9c | 36.3±3.6b | 17.6±0.9d | 7.9±2.3e | 19.4 |
| Manganese | 2.5±0.5d | 6.9±0.8b | 4.4±0.3c | 10.3±0.7a | 4.9±0.28c | 0.1±0.1e | 0.10 |
| Zinc | 20.9±1.3c | 23.3±2.7a | 29.7±1.4b | 48.3±2.5a | 8.7±0.75d | 20.8±5.0c | 41.8 |

Note: ** means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [19].

Table 5: Mineral composition (ppm/SD) of two formulations of cooked Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component | BMB1 | BMB2 | IFB1 | IFB2 | BBB | GP | GB* |
|-----------|------|------|------|------|-----|----|-----|
| Calcium | 267.3±15.2* | 1068.2±89.3* | 297.0±15.2* | 2165.0±69.5* | 1004.5±50.4* | 139.1±87.3* | 240.0 |
| Magnesium | 235.3±6.3* | 446.7±31.7* | 140.8±6.5* | 827.0±23.1* | 410.0±9.6* | 239.2±17.7* | 200.0 |
| Phosphorus | 2315.0±79.4* | 3120.0±182.5* | 1513.3±28.8* | 2143.3±60.2* | 1425.0±47.2* | 2146.7±21.8* | 1940.0 |
| Potassium | 3378.3±367.1* | 3056.7±287.3* | 3590.0±61.0* | 6753.3±326.2* | 3270.0±120.7* | 4220.0±834.9* | 3040.0 |
| Sodium | 4135.0±355.8* | 4186.7±262.0* | 5666.7±213.9* | 4249.0±219.3* | 3690.0±216.4* | 1277.8±1586.4* | 750 |
| Copper | 4.9±0.5* | 2.8±0.4* | 4.5±0.6 | 3.1±0.4 | 2.9±0.2 | 1.4±0.2* | 0.8 |
| Iron | 60.0±5.5* | 47.4±3.0 | 27.0±1.4 | 42.6±3.2 | 20.7±1.0 | 10.8±2.6 | 24.8 |
| Manganese | 3.0±0.3* | 9.0±0.8 | 5.0±0.4 | 12.0±0.8 | 5.5±0.3 | 0.2±0 | 0.11 |
| Zinc | 25.6±2.0 | 30.3±2.8 | 34.2±1.8 | 56.8±3.4 | 9.9±0.9 | 28.2±5.5 | 62.5 |

Note: ** means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [20].
for males and females at 1.2 and 1.1 mg/d, respectively; although one serving of GP would also meet the thiamin (B₁) RDA for adults as well. Furthermore, one serving of cooked GB or GP would supply approximately 36.2 to 56.3% of the adult RDA for niacin (B₃) [40]. One serving of cooked GB or GP would supply approximately 14.9 to 20.0% of the adult RDA for pantothenic acid (B₅), while PBMA would supply between 4.8 to 9.1% of the adult RDA for pantothenic acid (B₅) [40].

Table 6: Vitamin composition (mcg/g±SD) of two formulations of cooked Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component            | BMB1     | BMB2     | IFB1     | IFB2     | BBB      | GP       | GB*      |
|----------------------|----------|----------|----------|----------|----------|----------|----------|
| Vitamin A            | <0.3     | <0.3     | <0.3     | <0.3     | <0.3     | <0.3     | <0.3     | 0.04     |
| Vitamin D₃           | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | 0.07     |
| Vitamin D₅           | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | NA       |
| Vitamin E            | 21.7±4.5 | 17.1±3.4 | 33.9±5.9 | 71.0±4.9 | 15.9±1.7 | 5.1±2.4  | 1.7      |
| Vitamin K₃           | 0.2±0.02 | 0.1±0.03 | <0.04    | <0.04    | <0.06±0.01 | 0.04    | 0.02     |
| Thiamin (B₁)         | 0.6±0.5  | 0.5±0.1  | 182.3±7.5| 190.5±19.5| 1.1±1.1  | 3.3±1.0  | 0.43     |
| Riboflavin (B₂)      | 1.2±0.1  | 1.1±0.2  | 3.8±0.3  | 2.9±0.2  | 2.5±0.3  | 2.5±0.5  | 1.51     |
| Niacin (B₃)          | 3.5±0.3  | 5.6±0.4  | 52.6±4.9 | 51.7±4.9 | 8.4±0.4  | 56.0±13.7| 42.3     |
| Pantothenic Acid (B₅)| 3.6±0.3  | 1.8±0.2  | 3.4±0.3  | 2.0±0.2  | 3.7±0.3  | 8.1±2.0  | 5.0      |
| Pyridoxine Free Base (B₆)| 0.4±0.1  | 0.5±0.1  | 2.9±0.2  | 4.9±0.2  | 1.0±0.1  | 3.6±1.2  | 3.2      |
| Biotin (B₇)          | 0.06±0.01| 0.05±0.00| 0.04±0.01| 0.1±0.02 | 0.05±0.01| 0.04±0.01| NA       |
| Folic acid (B₉)      | NT       | 0.3±0.04 | NT       | 1.1±0.1  | NT       | 0.05**   | 0.1      |

Note: ** Means within a row with different subscripts differ statistically (P<0.05).

*Data collected from USDA nutrient database [19].
NA: Not Available; NT: Not tested.
<: Sample levels were not identified above the analysis thresholds for detection.

Table 7: Vitamin composition (mcg/g±SD) of two formulations of cooked Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

| Component            | BMB1     | BMB2     | IFB1     | IFB2     | BBB      | GP       | GB*      |
|----------------------|----------|----------|----------|----------|----------|----------|----------|
| Vitamin A            | <0.3     | <0.3     | <0.3     | <0.3     | <0.3     | <0.3     | 0.03     |
| Vitamin D₃           | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | NA       |
| Vitamin D₅           | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | <0.001   | NA       |
| Vitamin E            | 26.6±6.7 | 18.4±4.3 | 38.8±2.1 | 80.3±4.8 | 19.6±0.4 | 5.7±1.8  | 1.2      |
| Vitamin K₃           | 0.3±0.03  | 0.2±0.03  | <0.04    | <0.04    | 0.06±0.01 | <0.04    | 0.02     |
| Thiamin (B₁)         | 0.3±0.1  | 0.7±0.1  | 197.3±9.7 | 206.5±13.3| 0.7±0.4  | 4.0±1.3  | 0.5      |
| Riboflavin (B₂)      | 1.6±0.2  | 1.5±0.1  | 4.4±0.2  | 3.2±0.2  | 3.0±0.2  | 3.1±0.4  | 1.8      |
| Niacin (B₃)          | 4.3±0.3  | 6.0±0.5  | 62.2±3.0 | 58.2±2.9 | 10.5±0.1 | 80.0±6.9 | 51.0     |
| Pantothenic Acid (B₅)| 4.0±0.2  | 3.0±0.4  | 3.5±0.2  | 2.1±0.2  | 3.9±0.3  | 8.9±1.3  | 6.6      |
| Pyridoxine Free Base (B₆)| 0.5±0.1  | 0.5±0.1  | 3.1±0.2  | 5.6±0.5  | 1.2±0.1  | 3.0±0.5  | 3.7      |
| Biotin (B₇)          | 0.1±0.01  | 0.1±0.0  | 0.1±0.0  | 0.2±0.01 | 0.07±0.01 | 0.05±0.01| NA       |
| Folic acid (B₉)      | NT       | 0.4±0.1  | NT       | 1.1±0.1  | NT       | 0.06**   | 0.1      |

Note: ** Means within a row with different subscripts differ statistically (P<0.05).

*Data collected from USDA nutrient database [20].
NA: Not Available; NT: Not tested.
<: Sample levels were not identified above the analysis thresholds for detection.

While research has demonstrated that some B-vitamins may have poor thermol stability, photo stability, and evaporation loss during storage and cooking, the fluorometric and microbial analysis performed in this study did not demonstrate high B-vitamin loss [55-58]. Nonetheless, the bioavailability of B-vitamins can be impacted by multiple factors [59]. Niacin (B₃) is usually chemically bound when found in plant materials, while thiamin (B₁) and pyridoxine (B₆) can undergo Maillard reactions which may affect bioavailability [60,61]. Moreover, the crystalline nature of thiamin (B₁) supplements and the presence of other minerals may impact bioavailability [62]. Considering the high concentrations of thiamin (B₁) and pyridoxine (B₆) in IFB1 and IFB2 products, cooking may reduce the availability of these
vitamins through the Maillard reaction [63]. Furthermore, high thiamin \((B_1)\) concentrations may inhibit riboflavin \((B_2)\) and pyridoxine \((B_6)\) absorption [64,65]. Copper, calcium, iron, and other minerals, in high concentrations may also antagonize B-vitamin absorption, although the effect of other antagonists, food matrix, and other factors impacting B-vitamin absorption would have to be researched [66].

**Fatty acid analysis**

Results from fatty acid analysis for previous and current formulations of raw and cooked samples are reported in tables 8 and 9, respectively. Raw and cooked PBMA were below the detection limit \((<0.01mg/g)\) for cholesterol levels, while cooked GP contained approximately 0.86mg/g. A major finding of the fatty acid analysis was that the total saturated fatty acid content of PBMA ranged between 43 and 44% for BMB2 and IFB2, while GP and GB ranged between 39 and 42% of the total fatty acids. Additionally, total monounsaturated fatty acid content of BMB2 and IFB2 was similar to GB (at less than 55%), whereas GP contained the most total polyunsaturated fatty acid content (approximately 24%). As previously mentioned, not only did crude fat content not differ \((P<0.05)\) between PBMA and GP, but total saturated fatty acid content in BMB2 and IFB2 was similar to GP and GB. Furthermore, raw and cooked GP and GB had higher numerical values for total mono-unsaturated and polyunsaturated fatty acids than BMB2 and IFB2.

Palmitic \((C16:0)\) and stearic \((C18:0)\) acid compositions were greater \((P<0.05)\) in GP than in the PBMA, although, oleic \((C18:1)\) acid content in BMB2 and IFB2 was greater \((P<0.05)\) than GP. Substantial increases \((P<0.05)\) in oleic \((C18:1)\) and linoleic \((C18:2)\) acid content from IFB1 to IFB2 may be a result of sunflower oil which was used in addition to coconut oil in the IFB2 formulation [67]. Cocoa butter was substituted in the BMB2 formulation for sunflower oil, which may explain the increase \((P<0.05)\) in oleic \((C18:1)\) content from BMB1 and decrease \((P<0.05)\) in linoleic acid \((C18:2)\) content from BMB1 [67,68].

Linoleic \((C18:2)\) and a-linolenic \((C18:3)\) acids, cannot be synthesized \textit{de novo}\ and are essential for human growth and development [69].The BMB1 and BBB products proved to be a potentially excellent source of these essential fatty acids compared to the other PBMA. Conversely, GP was an excellent source of linoleic \((C18:2)\) and arachidonic \((C20:4)\) acid compared to BMB2 and IFB2. Fatty acids are readily absorbed in the human body, but the presence of fibrous materials and various thickeners and binders incorporated into PBMA formulations may inhibit fatty acid absorption [70,71].

**Amino acid analysis**

Results from amino acid analysis for previous and current formulations of raw and cooked samples are reported in tables 10 and 11, respectively. Total essential amino acid content of raw BMB1, BMB2, and IFB1 ranged between 70 and 76mg/g while total essential amino acid content of raw GP and GB ranged between 66 and 68mg/g. Histidine, methionine, and lysine concentrations were greater \((P<0.05)\) in GP compared to PBMA. Conversely, GB was numerically greater than the PBMA and GP in histidine, methionine, and lysine content, which may confer preferable anabolic capacity for the ABMs compared to the PBMA [72]. Cooked BMB2 was greater \((P<0.05)\) than GP in isoleucine and phenylalanine content, probably as pea and mung bean protein isolates (used in BMB2 formulation) are excellent sources of these essential amino acids [73,74]. Finally, IFB2 and BBB was either numerically or statistically less than \((P<0.05)\) GP in each essential amino acid; and were also less than \((P<0.05)\) BMB2 in each essential amino acid assessed, except threonine and tryptophan.

The PBMA were either numerically comparable to or statistically greater than \((P<0.05)\) ABM for most non-essential amino acid profiles. BMB2 was greater than \((P<0.05)\) G Pin arginine, aspartic acid, glutamic acid, serine, and tyrosine content. Conversely, GB was numerically greater than the PBMA and GP in glycine and alanine content. Substantial increases \((P<0.05)\) in glutamic acid, glycine, serine, and proline were observed from BMB1 to BMB2 formulation, probably due to the inclusion of and mung bean protein isolates which are relatively high in the aforementioned amino acids [74,75]. In contrast, IFB2 and BBB were either numerically comparable to or statistically less \((P<0.05)\) than GP and BMB2 in most non-essential amino acids assessed. Substantial decreases \((P<0.05)\) in nearly each non-essential amino acid were observed from IFB1 to IFB2, as wheat protein isolates are exceptionally high in glutamic acid, proline, and potato protein is high in many of the other non-essential amino acids [72].

The presence of fibrous material and anti nutrient components in some plant products may inhibit protein digestibility of PBMA [76]. The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is a simple evaluation of protein quality related to the amount of the first limiting amino acid in a test protein to the human metabolic requirement of that corresponding amino acid. The PDCAAS of soy, wheat, pea, mung bean, and bean proteins are 95, 96, 88, 76, and 78%, respectively, while the PDCAAS score for pork and beef has a score of 100% [77-79]. However, it has been suggested that PDCAAS digestibility scores fail to account for anti nutrient factors present in plant materials and substantially over-estimate the digestibility of many plant proteins [80]. Plant-based proteins have been reported to have depressed bioavailability compared to ABMs, although the absorption of amino acids from PBMA in comparison to ABM should be further evaluated [81].

**Conclusion**

The PBMA were numerically comparable to GP in crude protein and crude fat content. In general, the PBMA were high in several minerals, potentially competing for absorption, although this effect would have to be further researched. The IFB2 was high in thiamin \((B_1)\), niacin \((B_3)\), pyridoxine \((B_6)\), biotin \((B_7)\), and folates \((B_9)\). However, high thiamin \((B_1)\) concentrations may inhibit the absorption of riboflavin \((B_2)\) and pyridoxine \((B_6)\). Crude fat and total saturated fatty acid content were similar between BMB2, IFB2, and GP. In particular, BMB2 and IFB2 more closely resembled the fatty acid profile of GP, than the previous formulations of BMB1 and IFB1. The PBMA were generally comparable to GP in most essential amino acid profiles, although high lysine and methionine content. Overall, PBMA were generally comparable to ABM in many of the nutrient profiles assessed. However, it is important to investigate further the bioavailability of these nutrients, when incorporated into a PBMA food matrix and exposed to different anti nutrient compounds and antagonistic interactions.

**Acknowledgement**

Funding for this research was provided in part by The Beef Checkoff, Denver, CO, and in part by the National Pork Checkoff, Clive, IA.
| Component        | BMB1   | BMB2   | IFB1   | IFB2   | BBB    | GP     | GB*    |
|------------------|--------|--------|--------|--------|--------|--------|--------|
| Cholesterol      | <0.01±0.0 a | <0.01±0.0 a | <0.01±0.0 a | <0.01±0.0 a | <0.02±0.0 a | 0.7±0.1 a | 0.7 ±0.1 a |
| C18:0            | 1.2±0.2 b  | ND     | 8.4±0.3 d | ND     | ND     | 0.2±0.5 b | ND     |
| C10:0            | ND     | ND     | 7.0±0.8 d | ND     | ND     | 0.1±0.01 a | ND     |
| C12:0            | 3.5±0.8 a  | ND     | 46.8±1.1 d | ND     | ND     | 0.01±0 a  | 0.1±0.02 a | 0.1     |
| C14:0            | ND     | 5.2±0.9 d | 22.8±1.1 d | 5.9±0.8 d | 0.1±0 b  | 1.4±0.1 c | 3.3     |
| C16:0            | 7.4±0.6 d | 11.8±0.5 a | 2.0±0.3 d | 11.8±1.0 d | 8.5±0.1 b | 23.9±0.7 d | 23.7    |
| C18:0            | 5.3±0.4 e | 8.4±0.5 a | 2.3±0.2 a | 7.9±1.0 a | 2.0±0.2 d | 12.8±1.3 a | 13.1    |
| C20:0            | 1.1±0.1 d | 18.4±0.4 b | 0.2±0.1 b | 18.7±1.6 b | 0.4±0 d  | 0.1±0.1 b | 0.1     |
| C16:1            | ND     | ND     | ND     | ND     | 0.4±0 c  | 2.5±0.2 b | 4.0     |
| C18:1            | 31.9±2.2 a | 53.8±1.2 a | 7.0±0.3 a | 53.6±1.5 a | 10.0±0.4 c | 33.4±2.9 a | 42.5    |
| C18:1 n7         | ND     | ND     | 0.03±0.07 a | ND     | 2.0±0.2 d | 4.6±0.4 a | 6.5     |
| C20:1            | 1.1±0.1 b  | ND     | 0.2±0.04 a | ND     | 0.2±0 b  | 0.6±0.04 a | 0.4     |
| C18:2            | 34.6±1.4 b | 5.2±0.1 b | 2.1±1.0 b | 2.4±0.3 b | 34.2±0.7 b | 14.4±1.4 b | 2.3     |
| C18:3            | 12.5±0.5 b | ND     | 0.7±0.1 b | ND     | 41.9±0.4 b | 1.8±0.6 b | 0.4     |
| C20:4            | ND     | 0.4±0.03 b | ND     | 0.1±0.1 b | ND     | 0.4±0 b  | 3.6±0.5 a | 0.2     |
| Total Saturated  | 19.6±2.6 a | 43.7±5.0 a | 89.9±14.8 a | 44.1±5.1 a | 11.0±2.7 a | 38.9±8.0 a | 42.0    |
| Total Monounsaturated | 33.0±15.3 | 53.8±28.1 | 7.2±3.4 | 53.6±28.0 | 10.6±4.7 | 36.5±15.6 | 55.2    |
| Total Polyunsaturated | 47.5±11.7 | 2.5±0.1 | 3.0±0.8 | 2.4±0.3 | 78.4±16.6 | 24.8±8.7 | 2.9     |

Table 8: Cholesterol content (mg/g±SD) and fatty acid (% total FA±SD) profile of previous and current formulations of raw Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

Note: **Means within a row with different subscripts differ statistically (P<0.05).

*Data collected from USDA nutrient database [19].

ND: Not Detected.

Table 9: Cholesterol content (mg/g±SD) and fatty acid (% total FA±SD) profile of cooked Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

Note: **Means within a row with different subscripts differ statistically (P<0.05).

*Data collected from USDA nutrient database [20].

ND: Not Detected.

*: Sample levels were not identified above the analysis thresholds for detection.
Table 10: Amino Acid composition (mg/g/SD) of two formulations of raw Beyond Meat Burger (BMB1 and BMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

Note: **Means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [19].
**Includes the following: arginine, histidine, cystine, glycine, tyrosine, and proline.
***Glutamic acid, alanine, and aspartic acid.

| Component   | BMB1          | BMB2          | IFB1          | IFB2          | BBB          | GP          | GB**        |
|-------------|---------------|---------------|---------------|---------------|--------------|-------------|-------------|
| Arginine    | 16.8±1.1*     | 20.2±0.9*     | 9.7±0.6*      | 13.8±0.6*     | 8.8±0.5*     | 15±0.1±1*   | 16.8*       |
| Cystine     | 2.7±0.2*      | 3.1±0.1*      | 7.1±0.6*      | 4.9±0.2*      | 3.0±0.5*     | 2.5±0.2*    | 2.7*        |
| Glutamic Acid| 33.6±1.7*     | 40.8±1.5*     | 80.9±5.0*     | 45.2±1.2*     | 34.7±5.2*    | 32±1.3±0    | 38.6        |
| Glycine     | 8.2±0.4*      | 9.9±0.5*      | 10.2±0.5*     | 8.6 ± 0.2*    | 5.3±0.4*     | 13.0±2.2*   | 17.5        |
| Proline     | 9.0±0.5*      | 11.0±0.4*     | 25.3±1.5*     | 10.1±0.4*     | 11.5±1.9*    | 11.1±1.2*   | 13.1        |
| Tyrosine    | 7.7±0.4*      | 10.1±0.5*     | 10.0±0.5*     | 7.8±0.2*      | 6.0±0.5*     | 7.4±0.5*    | 7.9         |
| Histidine   | 4.8±0.2*      | 5.9±0.3*      | 4.8±0.3*      | 4.7±0.2*      | 3.5±0.3*     | 7.6±0.6*    | 8.4         |
| Isolucine   | 9.8±0.5*      | 11.7±0.5*     | 10.7±0.6*     | 9.5±0.2*      | 6.9±0.5*     | 10.0±0.7*   | 11.4        |
| Leucine     | 17.2±0.8*     | 20.3±0.8*     | 19.5±1.0*     | 15.8±0.4*     | 12.4±0.9*    | 17.5±1.3*   | 20.1        |
| Lysine      | 14.5±1.0*     | 15.6±0.6*     | 8.5±0.3*      | 12.2±0.6*     | 7.5±0.4*     | 18.2±1.1*   | 21.3        |
| Methionine  | 1.8±0.3*      | 3±0.1*        | 3.8±0.2*      | 2.4±0.1*      | 3.3±0.2*     | 5.0±0.7*    | 6.6         |
| Phenylalanine| 10.9±0.6*    | 13.6±0.5*     | 13.6±0.6*     | 10.5±0.3*     | 8.1±0.6*     | 8.6±0.6*    | 10.0        |
| Threonine   | 7.4±0.4*      | 8.9±0.4*      | 8.0±0.4*      | 7.9±0.2*      | 5.7±0.3*     | 9.6±0.7*    | 10.0        |
| Tryptophan  | 2.0±0.2*      | 2.4±0.1*      | 2.6±0.1*      | 2.6±0.1*      | 1.8±0.2*     | 2.7±0.2*    | 1.3         |
| Valine      | 10.0±0.5*     | 12.8±0.5*     | 11.9±0.5*     | 9.9±0.2*      | 7.7±0.6*     | 10.7±0.9*   | 12.6        |
| Alanine     | 9.0±0.4*      | 11.1±0.5*     | 7.8±0.4*      | 9.2±0.3*      | 6.6±0.3*     | 14.0±1.4*   | 16.1        |
| Aspartic Acid| 23.9±1.3*     | 27.2±1.0*     | 15.6±1.0*     | 22.4±0.6*     | 15.2±0.8*    | 20.3±1.6*   | 23.2        |
| Serine      | 10.3±0.5*     | 12.3±0.6*     | 12.4±0.7*     | 10.0±0.4*     | 8.3±0.6*     | 8.6±0.7*    | 10.3        |
| Total essential | 78.2±5.1   | 94.2±5.7      | 83.2±5.1      | 75.4±4.3      | 56.8±3.1     | 90.7±4.8    | 101.7       |
Total conditionally-essential** 78.0±10.3 95.0±12.4 143.2±26.7 90.4±13.9 69.3±11.1 81.1±9.5 96.5
Total non-essential*** 43.1±7.0 50.6±7.6 35.7±3.4 41.7±6.2 30.1±3.9 42.9±5.0 49.6

Table 11: AA composition (mg/g±SD) of two formulations of cooked Beyond Meat Burger (BBMB1 and BBMB2), Impossible Foods Burger (IFB1 and IFB2), Black Bean Burger (BBB), 80/20 Ground Pork (GP), and 80/20 Ground Beef (GB).

Note: **Means within a row with different subscripts differ statistically (P<0.05).
*Data collected from USDA nutrient database [20].
**Includes the following: arginine, histidine, cystine, glycine, tyrosine, and proline.
***Glutamic acid, alanine, and aspartic acid.

Disclosure statement

The authors declare that they have no conflicts of interest. Use of trade names in this publication does not imply endorsement or criticism by Colorado State University of those or similar products not mentioned.

Mention of a proprietary product does not constitute a guarantee or warranty of the products by Colorado State University or the authors and does not imply its approval to the exclusion of other products that may also be suitable.

Data Availability

All data generated or analyzed during this research are included in this manuscript.

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