5.0 CHARACTERIZATION, SPECIFICATIONS, AND BATCH ANALYSES

Comprehensive characterization of the identity, quality, and composition of the company's cell lines, and harvested biomass was paramount to the safety evaluation procedure. Believer Meats verifies that the cell lines are derived from the correct species, that the cell type (*e.g*., fibroblast) displays characteristics that are defining features of the tissues from which the cells are derived, respond to differentiation cues in a predictable manner resulting in differentiation towards cell types that are characteristic of meat (*e.g*., myoblast, adipocyte), and that these defining characteristics are stable and do not change over the expected life-span of the cell line. Believer Meats also has demonstrated that the cell lines do not display unstable genotypes through karyotyping and demonstration of stable cell PD times. Cells with unstable genomes can exhibit inefficient growth (*e.g*., excess consumption of glucose) and produce unpredictable phenotypic changes, and while not implicitly a food safety concern, as poultry cells do not have the genetic capacity to produce toxic or other undesirable substances, represent changes that are not conducive to efficient use in food production. Genetic instability of a cell line leading to uncontrolled growth patterns could also manifest in unexpected changes in nutrient composition that could be a food safety concern. Currently, Believer Meats' cell lines have been demonstrated to display stable PD times in excess of 600 PDs. Studies demonstrating the species identity and phenotypic and genetic stability of the FMT-SCF-4 cell line are outlined in the sections below.

Believer Meats has established food-grade specifications for production lots harvested from the bioreactor. Cultured chicken cells must meet general proximate limits for pH, moisture, carbohydrates, fat and ash and limits for environmental (*e.g.*, heavy metals) and microbial contaminants (spoilage organisms and human food safety pathogens) that may originate from the production process inputs. Batch analyses demonstrating compliance of multiple production lots are provided. Each production lot is produced from a newly sterilized bioreactor, seeded from the MWCB.

Finally, complete compositional testing of the harvested biomass is provided, demonstrating that the nutritional composition of the biomass falls within the range of conventional chicken meat products or other commonly consumed foods in the diet. Concentrations of nutrients at levels of toxicological concern were not observed. Analyses for residues of various biologically active media components that are of food safety concern are discussed below.

5.1 Characterization of the Cell Line

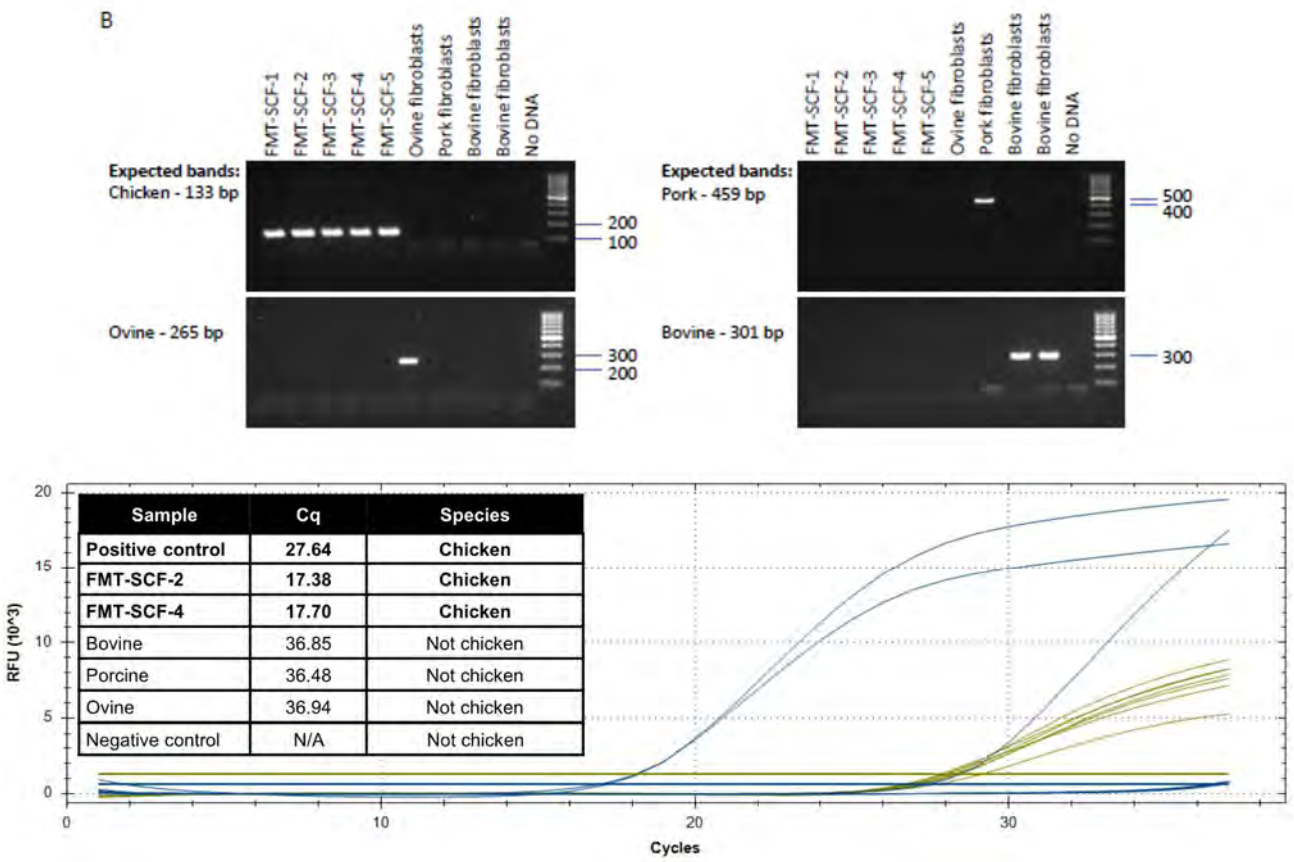
5.1.1 Confirmation of Species Identity

Believer Meats carried out extensive genomic analysis on the FMT-SCF-4 production cell line. This included DNA, RNA, and protein analysis validating species origin and cellular identity. Data were published by Pasitka *et al*. (2023) and was made available online (GSE169291).

Believer Meats used the mitochondrially encoded cytochrome B gene (MT-CYB) to determine species identity in its MCBs due to its sequence variability across different phylogeny (Castresana, 2001). Five different cell lines of cultured chicken fibroblasts were analyzed by PCR. Note that SCF-1, SCF-2, SCF-3, and SCF-5 are MCBs that did not continue into development of a MWCB like SCF-4. Following DNA amplification, gel electrophoresis was performed with appropriate positive and negative controls. Results from this analysis indicate that all MCB cells are solely derived from chicken (*Gallus gallus*) as indicated in Figure 5.1.1-1 (top panel).

Believer Meats also utilized Thermo Scientific™ RapidFinder™ Chicken ID Kit to validate its secondary MCBs. The kit uses quantitative real-time PCR to detect DNA in both raw and processed chicken in food products and provides sensitivity down to 0.01% chicken DNA. The kit positive control is 0.1% chicken DNA. MCB samples were negative to other species and showed a signal of 10 ΔC_t above the positive control as shown in Figure 5.1.1-1 (bottom panel).

Figure 5.1.1-1 Confirmation of Species Identity



Sample	Cq	Species
Positive control	**27.64**	**Chicken**
FMT-SCF-2	**17.38**	**Chicken**
FMT-SCF-4	**17.70**	**Chicken**
Bovine	36.85	Not chicken
Porcine	36.48	Not chicken
Ovine	36.94	Not chicken
Negative control	N/A	Not chicken

bp = base pairs; FMT = Future Meat Technologies; N/A = not applicable, RFU= relative fluorescent units

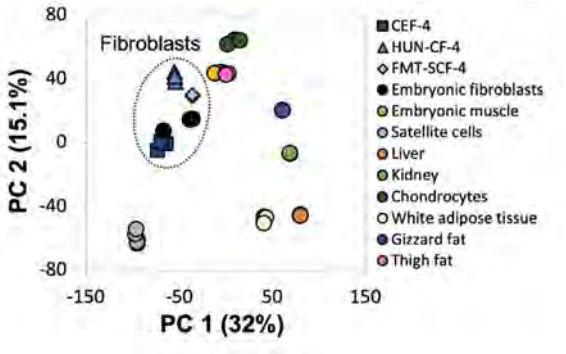
Figure 5.1.1-1 Bottom panel depicts the results of qPCR analysis using the Thermo Scientific™ RapidFinder™ Chicken ID Kit to detect chicken DNA. The kit includes two multiplex reactions: an internal control for general gene amplification (green lines) and a specific primer pair for chicken DNA (blue lines). The amplification quantification chart shows a strong positive signal for FMT-SCF-2 and 4 cell lines (Cq=~17). The kit contains a positive control with a Cq of =~27. As per the manufacturer's instructions, any sample with a Cq value higher than Cq=positive control+3.32 cycles is considered negative for chicken DNA.

5.1.2 Confirmation of Fibroblast Identity

To further characterize the cellular identity of the cell lines, Believer Meats carried out RNA-Seq analysis on the primary cell isolates (CEF-4), the immortalized adherent cell lines (HUN-CF-4), and suspension-adapted lines (FMT-SCF-4). RNA-Seq data were downloaded for various chicken tissues including liver, kidney, muscle, satellite cells (muscle stem cells), chondrocytes (cartilage cells), gizzard fat, thigh fat, white adipose tissue, and embryonic fibroblasts. RNA-Seq data were normalized and separated *via* principal component analysis. Figure 5.1.2-1 demonstrates that Believer Meats' primary cell isolates (CEF-4) cluster together with chicken embryonic fibroblasts, validating the company's cell source. The data further shows that the

immortal cell lines (HUN-CF-4) and suspension-adapted line (FMT-SCF-4) cluster with chicken embryonic fibroblasts, demonstrating that Believer Meats' spontaneous immortalization process did not change the core transcriptional identity of the cells.

Figure 5.1.2-1 RNA-Seq Analysis of Immortalized and Suspension-adapted Cell Lines as Compared to RNA-Seq Data for Various Chicken Tissues



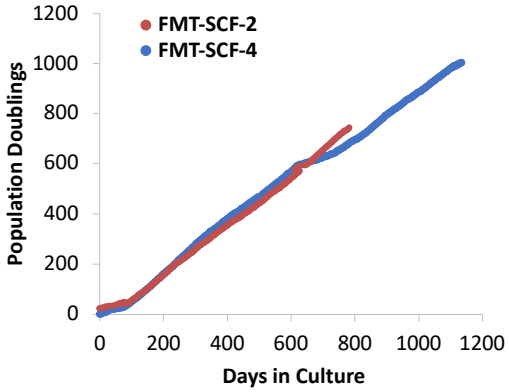
5.1.3 Stability of Identity Over Time

Believer Meats' production process relies on extensively characterized genetically stable cell lines of spontaneously immortalized fibroblasts grown in suspension. While genetic stability of therapeutic proteins is defined by the stability of their sequence and biological function, the genetic stability of cultured meat cell lines is defined by the stability of their karyotype. Genetic stability of Believer Meats' cell lines has been evaluated and details may be found in the peer-reviewed publication (Pasitka *et al.*, 2022). In summary, the normal macrochromosome distribution demonstrates karyotype stability for over 500 PDs, and single nucleotide variation (SNV) analysis of *TP53* in FMT-SCF-4 and its respective primary chicken fibroblasts showed no mutation occurs in *TP53* gene demonstrating unaltered ability to carry out DNA repair (Pasitka *et al*., 2023).

5.1.3.1 Growth Rate Stability

To demonstrate long term functional stability of the FMT-SCF-4 production cell line, Believer Meats tracked the growth rates of the cell lines for over 800 days (see Figure 5.1.3.1-1). The cell line showed stable doubling time and morphology for over 800 PDs.

Figure 5.1.3.1-1 Growth Rate Stability of Believer Meats' Cell Lines Tracked for Over 1,100 Days

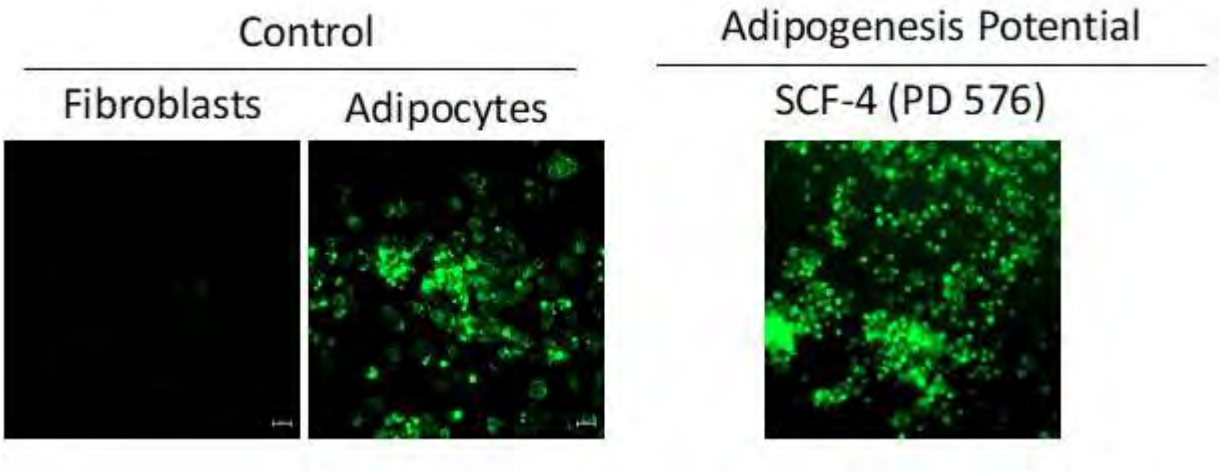


5.1.3.2 Cell-Line Stability of Differentiation Potential

Believer Meats has developed a proprietary method to differentiate cultured fibroblasts to cultured adipocytes using lipids. Detailed mechanism and cells characterization are published in Pasitka *et al*. (2023). In brief, Believer Meats identified phosphatidylcholine A, the major component of lecithin as a PPARγ agonist. Addition of a food-grade mixture of lecithin and fatty acids induces the rapid accumulation of lipid droplets in cultured fibroblasts, producing pre-adipocyte cells rich in flavor and aroma carrying phospholipids. For simplicity, the differentiated cells are defined as cultured adipocytes.

To demonstrate the long-term maintenance of adipogenic potential the FMT-SCF-4 production cell line, Believer Meats carried out differentiation at population day 576 using lecithin and fatty acids (see Figure 5.1.3.2-1). The FMT-SCF-4 underwent efficient lipid accumulation with over 85% of the cells accumulating lipids even at PD 576 representing 1.6 years of continuous growth.

Figure 5.1.3.2-1 Stability of Differentiation Potential Past 500 PDs

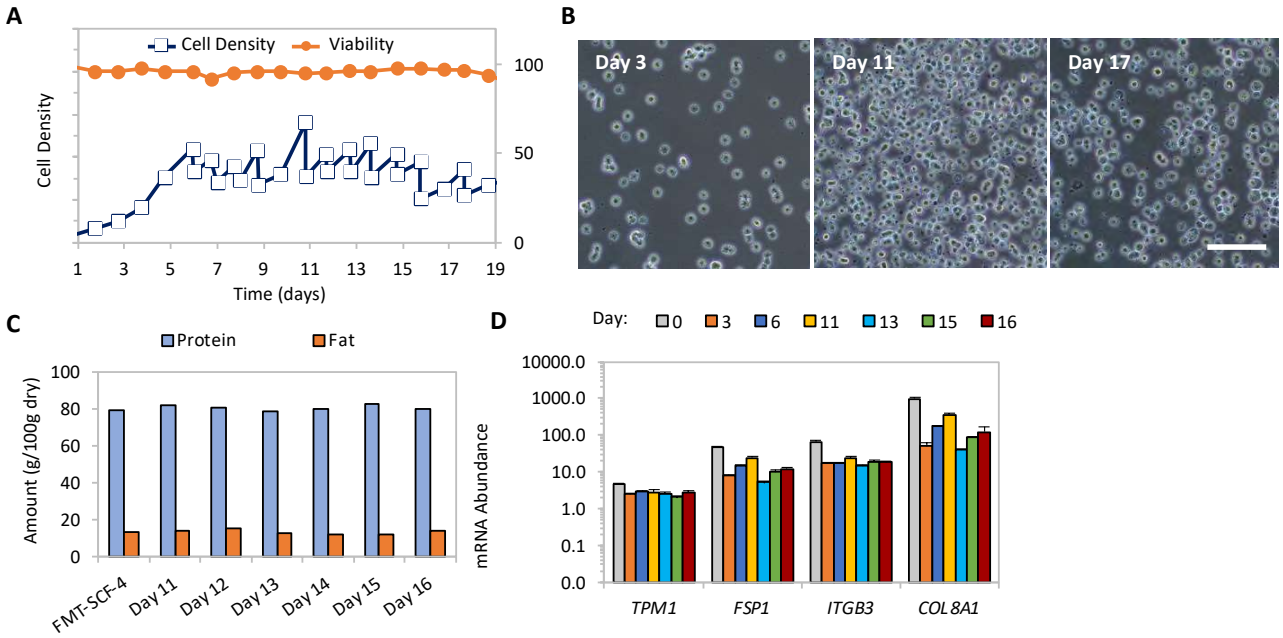


5.1.3.3 Cultured Meat Identity and its Stability in Continuous Production

Believer Meats uses the tropomyosin expression as a phenotypic identity marker for the cell-line. Tropomyosin is a structural protein encoded by the *TPM1* gene that is highly conserved and widely distributed with actin-binding proteins involved in the contractile system of striated and smooth muscles as well as the cytoskeleton of non-muscle cells (Matsuda *et al*., 1983). Expression of *TPM1* was demonstrated in MCB and WCB cells by quantitative real-time polymerase chain reaction (qRT-PCR). Although the expression TPM1 has no direct implications to the safety of the cell-line, it is used by Believer Meats as a phenotypic measure of cell line stability. Other markers characteristic of chicken fibroblasts (*e.g.*, FSP1, COL8A1) could also be used. The expression of TPM1 was also used in previous cell-culture consultations as a cell-line identity marker (See Section 5.2 of CCC0002).

To demonstrate the stability of *TPM1* expression in continuous culture, Believer Meats carried out a 19-day continuous harvest of FMT-SCF-4 production cell line from a bioreactor working under perfusion. Cellular morphology remained stable across 12 consecutive harvests. The composition of protein and fat in the biomass remained stable throughout the harvests. Finally, the harvested biomass was quantified by quantitative real-time polymerase chain reaction (qRT-PCR), showing persistent expression of *TPM1* and other fibroblast-specific markers during the extended operation.

Figure 5.1.3.3-1 Cultured Fibroblasts Identity and Stability in Continuous Production



qRT-PCR = quantitative real-time polymerase chain reaction.

(A) Cell density and viability tracked for 19 days operation in bioreactor. Cultured meat biomass harvests were carried out from Day 6 through 19 of the operation. **(B)** Phase images of harvested cells. Cell morphology is stable throughout the process. Scale bar 50 µm. **(C)** Nutritional analysis of harvested biomass showing persistent protein and fat composition. **(D)** qRT-PCR showing stable expression of chicken meat marker Tropomyosin 1 (*TPM1*), as well as fibroblast specific protein 1 (*FSP1*), integrin B3 (*ITGB3*), and collagen 8A1 (*COL8A1*), which identify chicken fibroblasts.

5.2 Specifications for Harvested Chicken Fibroblasts

Food-grade product specifications have been established for cultured chicken fibroblasts, including specification limits for proximate parameters, heavy metals, and microbiological contaminants (see Table 5.2-1). All methods of analysis are validated and internationally recognized.

Table 5.2-1 Product Specifications for Cultured Chicken Fibroblasts

Specification Parameter	Limit	Method of Analysis
Proximate Parameters		
Protein (dry basis) (%)	>65	Internal, based on AOAC 976.05, 950.36, 991.3
Moisture (%)	>95	AOAC 950.46
Ash (%)	>2.5	AOAC 923.03
Fat (hydrolysis) (%)	>10	Based on Nestle LI 00.527-1
Carbohydrates (%)	<10	Calculated [a]
Heavy Metals		
Lead (mg/kg)	<0.05	ICP-MS
Arsenic (mg/kg)	<0.01	ICP-MS
Cadmium (mg/kg)	<0.01	ICP-MS
Mercury (mg/kg)	<0.01	ICP-MS

Table 5.2-1 Product Specifications for Cultured Chicken Fibroblasts

Specification Parameter	Limit	Method of Analysis
Microbiological Parameters		
Total plate count (CFU/g)	<5,000	SI 885-02
Yeast (CFU/g)	<100	SI 885-08
Molds (CFU/g)	<200	SI 885-08
Escherichia coli TBX (CFU/g)	<10	SI 885-12
Salmonella sp.	Negative in 25 g	ISO 6579
Enterobacteriaceae (CFU/g)	<100	ISO 21528-2
Tropomyosin Expression (Cq)[b]	<26	qRT-PCR

AOAC = Association of Official Analytical Collaboration; CFU = colony-forming units; ICP-MS = inductively coupled plasma mass spectrometry; ISO=International Organization for Standardization; qRT-PCR=quantitative real-time polymerase chain reaction; SI= Israeli Standard; TBX=tryptone bile glucuronide

[a] Calculated: Carbs = 100 - (ash + moisture + protein + fats + dietary fibers).

[b] Quantitative Real Time PCR using 50 ng mRNA

5.3 Batch Analysis

Three non-continuous batches of cultured cells were analyzed for compliance with the specification requirements for CPM (see Tables 5.3-1). Production of the CPM lots was carried out using three separate vials from the MWCB and were performed in a non-continuous process where a newly sterilized bioreactor and fresh media were used for each production run to demonstrate the robustness of the quality controls used during the production process. The production runs were performed in bioreactors supported by media rejuvenation (see Figure 4.4-1). The results demonstrate that the manufacturing process, as described in Section 4.0, produces a consistent product that meets the established product specifications.

Table 5.3-1 Results of Analysis for Three Non-Continuous Batches of Cultured Chicken Fibroblasts

Specification Parameter	Specification Limit	Batch No.		
		1	2	3
Protein (%DMB)	>65	71.5	66.1	73.3
Moisture (%)	>95	96.45	95.6	96.39
Ash (%DMB)	>2.5	7.5	7.6	8.6
Fat (hydrolysis) (%DMB)	>10	18.2	24.0	11.6
Carbohydrates (%)	<10	<0.10	<0.10	0.23
Lead (mg/kg)	<0.05	<0.005	<0.005	0.011
Arsenic (mg/kg)	<0.01	<0.01	<0.01	<0.01
Cadmium (mg/kg)	<0.01	<0.003	<0.003	<0.005
Mercury (mg/kg)	<0.01	<0.003	<0.003	<0.005
Total plate count (CFU/g)	<5,000	<10	<10	10
Yeast (CFU/g)	<100	<10	<10	<10
Molds (CFU/g)	<200	<10	<10	<10
Escherichia coli TBX (CFU/g)	<10	<10	<10	<10
Salmonella sp. (CFU/25 g)	Negative	Negative	Negative	Negative
Enterobacteriaceae (CFU/g)	<100	<10	<10	<10
Tropomyosin Expression (Cq)	< 26	20.3	21.7	20.6

Table 5.3-1 Results of Analysis for Three Non-Continuous Batches of Cultured Chicken Fibroblasts

Specification Parameter	Specification Limit	Batch No.		
		1	2	3

CFU = colony-forming units; LOQ = limit of quantitation.
* Value was below the LOQ when measured on a wet basis. In this case, the LOQ of 0.1% was utilized to calculate a value on Dry Matter Basis.

5.4 Nutritional Analysis as Compared to Conventional Chicken

Believer Meats has tested three non-continuous batches of its cultured chicken cells along with various conventional chicken products purchased in local supermarkets. Previous analyses evaluated by the U.S. FDA have indicated that established reference data may not capture "real life" situations; therefore, both the USDA databases and the contemporaneously obtained samples serve to provide comparators for purposes of compositional evaluation. As shown in the sections below, Believer Meats' cultured chicken cells are compositionally similar to ground chicken and chicken breast. As expected, the cultured chicken biomass displays a higher water content compared to conventional poultry meat products; however, cultured cells' protein and fat content ranged between that of conventional ground chicken and chicken breast when normalized to dry weight (see Figure 5.4-1 and Table 5.4-1).

The average cholesterol value in cultured chicken cells is 61 mg/100g (Table 5.4-3). These values are within the range of 42 mg/100g reported for USDA chicken breast and the Believer Meats recorded value of 87 mg/100g for commercially available ground chicken. This dietary intake of cholesterol also compares to commonly consumed foods in the diet. For example, a large grade A egg contains 411 mg of cholesterol (USDA ARS, 2019a). The 2015-2020 Dietary Guidelines for Americans does not include restrictions on the dietary intake of cholesterol (Soliman, 2018).

Figure 5.4-1 Nutritional Profile of Cultured Chicken Fibroblasts Compared to Ground Chicken

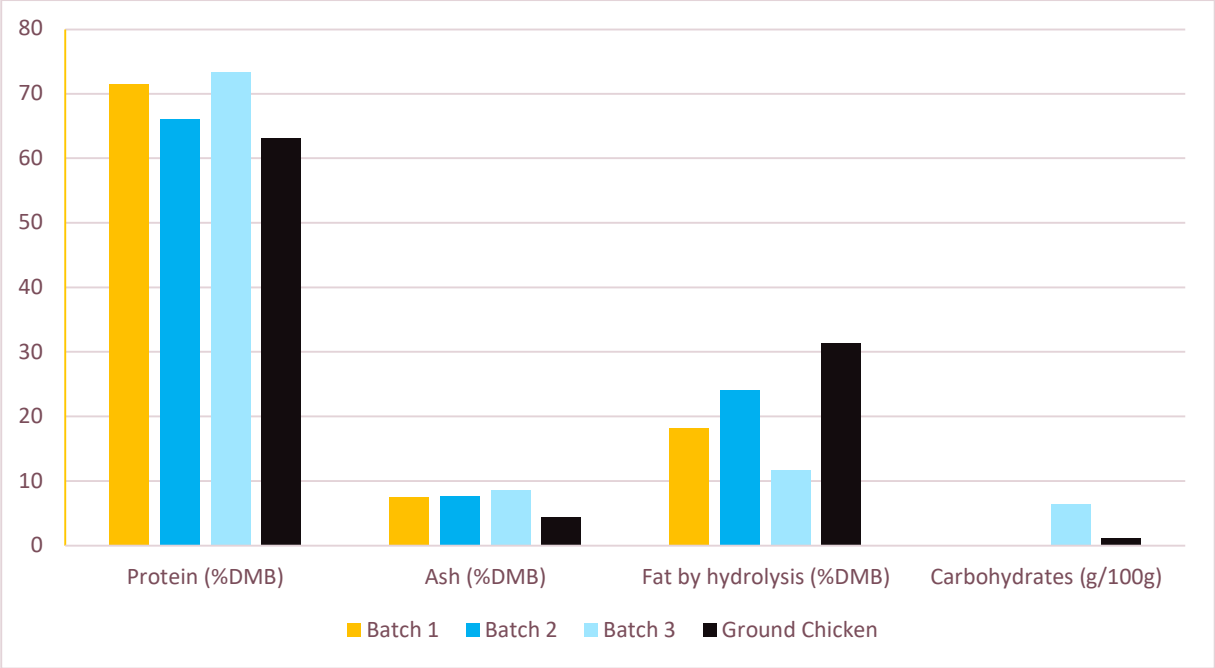


Table 5.4-1 Comparison of Proximate Results for Believer Meats Cultured Fibroblasts and Conventional Chicken Breast Products

Parameter (Dry matter basis)	Method	Cultured Chicken Fibroblasts			Chicken Products [A]		
		1	2	3	Ground Chicken	Chicken Breast	USDA Chicken
Moisture (%)	AOAC 950.46	96.45	95.6	96.39	71.0	74.9	73.9
Protein (% DMB)	In-house procedure, based on 976.05, 950.36, 991.3	71.5	66.1	73.3	63.2	85.4	86.2
Ash (% DMB)	AOAC 923.03	7.5	7.6	8.6	4.4	5.0	4.3
Fat (% DMB)	Based on Nestle LI 00.527-1	18.2	24.0	11.6	31.3	7.2	10
Carbohydrates (% DMB)	By difference	<2.8*	<2.3*	6.4*	1.1*	2.4*	0
Total dietary fiber (%)	In-house procedure MP 2135 rev. 6 based on AOAC 991.43	-	-	-	<0.5	<0.5	0
Energy (kcal/100g DMB)	Calculated: Energy (kcal) = 4*C+ 4*P+ 9*F+2*DF	461	490	423	538	415	120
Lipid Analysis	**Method**	**Cultured Chicken Fibroblasts**			**Chicken Products [A]**		
Saturated fat (g/100g)	AOAC 996.06	0.32	0.46	0.16	1.95	0.79	1.01
MUFA (g/100g)	AOAC 996.06	0.28	0.52	0.24	2.49	0.85	1.26
PUFA (g/100g)	AOAC 996.06	0.05	0.06	0.02	1.27	0.44	0.77
Cholesterol (mg/100g)	AOAC 994.10	57.2	67.8	56.8	93.5	73.4	42

AOAC = Association of Official Analytical Chemists; LOQ = limit of quantitation; MUFA = monounsaturated fatty acids; ND = not detectable; PUFA = polyunsaturated fatty acids.
*Value was below the LOQ when measured on a wet basis. In this case, LOQ (0.1%) was utilized to calculate values.
A - Presented as the average of 3 batches and includes data provided from commercial chicken products obtained from local marketplace.

The amino acid profile of 3 non-continuous batches of cultured chicken cells is presented in Table 5.4-2. When normalized on a dry matter basis (see Table 5.4-2 and Figure 5.4-2), the amino acid profiles of cultured chicken cells is almost identical to that of conventional chicken dry products.

Table 5.4-2 Amino Acid Profile for Cultured Chicken Fibroblasts

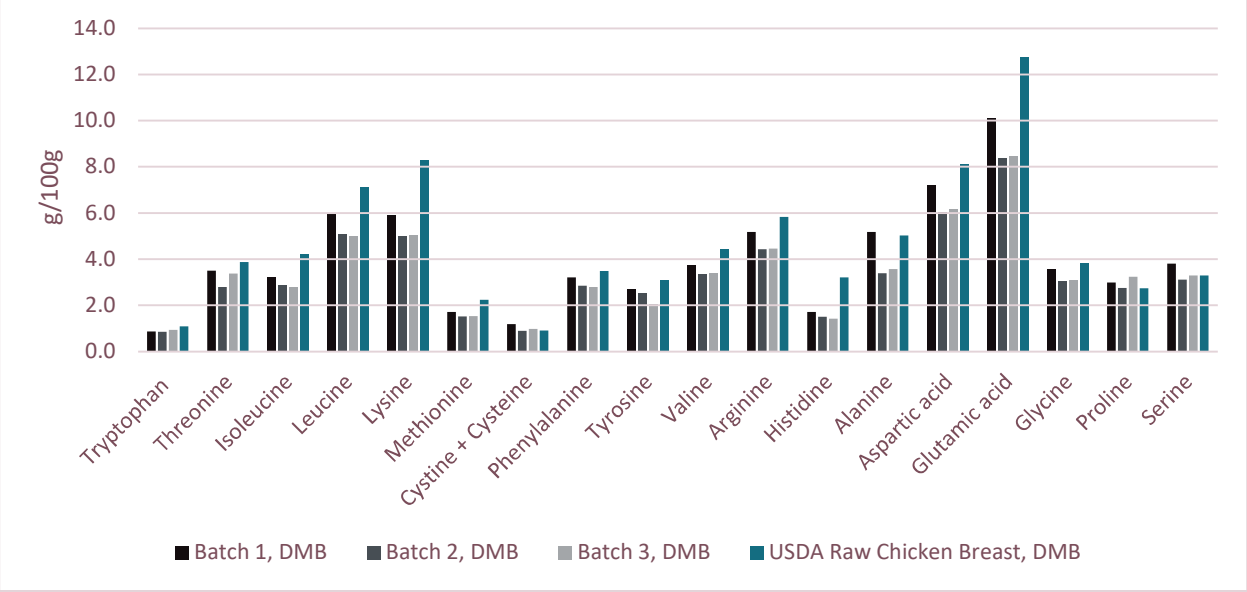
Amino Acids (g/100 g)	Cultured Chicken Fibroblasts (Wet Weight)			Cultured Chicken Fibroblasts (DMB)			USDA Raw Chicken Breast
	1	2	3	1	2	3	(DMB)
Tryptophan	0.0305	0.0377	0.0337	0.859	0.857	0.934	1.084
Threonine	0.124	0.123	0.122	3.493	2.795	3.380	3.870

Table 5.4-2 Amino Acid Profile for Cultured Chicken Fibroblasts

Isoleucine	0.114	0.127	0.101	3.211	2.886	2.798	4.215
Leucine	0.212	0.223	0.18	5.972	5.068	4.986	7.126
Lysine	0.21	0.219	0.182	5.915	4.977	5.042	8.276
Methionine	0.0609	0.067	0.0552	1.715	1.523	1.529	2.241
Cystine + Cysteine	0.0421	0.0393	0.0352	1.186	0.893	0.975	0.904
Phenylalanine	0.114	0.125	0.101	3.211	2.841	2.798	3.479
Tyrosine	0.096	0.111	0.075	2.704	2.523	2.078	3.103
Valine	0.133	0.147	0.122	3.746	3.341	3.380	4.444
Arginine	0.184	0.195	0.161	5.183	4.432	4.460	5.824
Histidine	0.0608	0.066	0.0514	1.713	1.500	1.424	3.215
Alanine	0.184	0.149	0.129	5.183	3.386	3.573	5.019
Aspartic acid	0.255	0.265	0.222	7.183	6.023	6.150	8.123
Glutamic acid	0.359	0.368	0.306	10.113	8.364	8.476	12.759
Glycine	0.126	0.133	0.112	3.549	3.023	3.102	3.816
Proline	0.106	0.121	0.117	2.986	2.750	3.241	2.739
Serine	0.135	0.137	0.119	3.803	3.114	3.296	3.287
Total amino acids	2.4953	2.653	2.2245	70.290	60.295	61.620	NR

DMB = dry matter basis; NR = not reported.

Figure 5.4-2 Amino Acid Profile of Cultured Chicken Fibroblasts Compared to Raw Chicken Breast (DMB)



USDA = United States Department of Agriculture; DMB = dry matter basis.

The fatty acid profile of three non-continuous batches of cultured chicken cells are presented below in comparison to data from commercial chicken breast analysed by Believer Meats. The fatty acid profile of cultured chicken cells is consistent with that of conventional chicken breast and ground chicken products, with cultured cells containing high levels of oleic acid (C18:1), palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2 all cis). Believer Meats also notes that the quantities of trans fatty acids (18:1 trans, 18:2 trans, and 18:3 trans) in cultured chicken cells is below that of the store-bought chicken products when compared on a wt/wt basis.

Table 5.4-3 Comparison of Fatty Acid Content for Cultured Chicken Fibroblasts and Conventional Chicken Breast Products

Fatty Acid	Cultured Chicken Cells (g/100g oil)			Wet Basis (g/100 g CF)			Store Bought Chicken (g/100 g Wet Basis)	
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Ground Chicken Sample[A]	Chicken Breast Sample[A]
C4:0	ND	0.035	ND	ND	ND	ND	0.0017*	0.0020*
C6:0	ND	0.046	ND	ND	ND	ND	ND	ND
C8:0	0.058	0.543	ND	0.0004	0.004	ND	0.0008*	ND
C10:0	0.207	0.442	ND	0.0014	0.003	ND	0.0046	0.0049*
C11:0	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	12.571	5.644	1.149	0.0842	0.038	0.008	0.0152	0.0045
C14:0	3.158	2.655	1.114	0.0212	0.018	0.007	0.0575	0.0171
C14:1 c9	ND	0.053	ND	ND	0.0004	ND	0.0133	0.0024
C15:0	0.157	0.065	0.117	0.0011	0.0004	0.001	0.0091	0.0026
C16:0	20.46	18.902	20.489	0.1371	0.127	0.137	1.9939	0.3902
C16:1 c9	2.657	4.192	3.988	0.0178	0.028	0.027	0.4676	0.0733
C17:0	0.102	0.055	0.06	0.0007	0.000	0.0004	0.0143	0.0032
C18:0	9.158	11.836	10.329	0.0614	0.079	0.069	0.6628	0.1853
C18:1 trans	1.841	1.803	1.946	0.0123	0.012	0.013	0.0460	0.0162
C18:1	36.203	39.521	46.916	0.2426	0.265	0.314	3.6294	0.6117
C18:2 trans	0.66	0.644	0.641	0.0044	0.004	0.004	0.0182	0.0050
C18:2 all cis-9,12	4.505	3.075	0.506	0.0302	0.021	0.003	1.7178	0.3142
C18:3 trans	0.478	0.72	0.77	0.0032	0.005	0.005	0.0095	0.0017
C18:3 all cis 6,9,12 G	ND	ND	ND	ND	ND	ND	0.0184	0.0033
C18:3 all cis 9,12,12 ALA	0.239	0.335	0.3	0.0016	0.002	0.002	0.1107	0.0165
C20:0	0.308	0.351	ND	0.0021	0.002	ND	0.0140	0.0032
C20:1 c11	0.133	1.637	1.743	0.0009	0.011	0.012	0.0425	0.0072
C20:2 all cis-11,14	ND	ND	ND	ND	ND	ND	0.0233	0.0094

Table 5.4-3 Comparison of Fatty Acid Content for Cultured Chicken Fibroblasts and Conventional Chicken Breast Products

Fatty Acid	Cultured Chicken Cells (g/100g oil)			Wet Basis (g/100 g CF)			Store Bought Chicken (g/100 g Wet Basis)	
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Ground Chicken Sample[A]	Chicken Breast Sample[A]
C20:3 all cis-8,11,14	0.051	0.274	0.732	0.0003	0.002	0.005	0.0177	0.0135
C20:3 all cis-11,14,17	0.19	ND	ND	0.0013	ND	ND	0.0022	0.0009
C20:4 all cis-5,8,11,14	0.306	0.226	0.163	0.0021	0.002	0.001	0.0657	0.0583
C20:5 all cis-5,8,11,14,17 EPA	0.336	0.493	0.385	0.0023	0.003	0.003	0.0035	0.0020
C21:0	ND	0.058	ND	ND	ND	ND	0.0080	0.0033
C22:0	1.683	2.088	2.728	0.0113	0.014	0.018	0.0211	0.0045
C22:1 n11	0.472	0.155	0.191	0.0032	0.001	0.001	0.0038	0.0030
C22:1 c11	0.154	0.446	0.349	0.001	0.003	0.002	0.0035	0.0012
C22:1 c13	0.41	0.127	ND	0.0027	0.001	ND	0.0007	0.0005*
C22:2 c-13,16	0.288	0.154	0.07	0.0019	0.001	0.0005	0.0022	0.0008
C22:4 all cis-7,10,13,16	ND	ND	ND	ND	ND	ND	ND	ND
C22:3 all cis-13,16,19	0.456	0.245	0.78	0.0031	0.002	0.005	0.0060	0.0079
C22:4 n6	ND	0.033	ND	ND	ND	ND	0.0182	0.0172
C22:5 all cis-4,7,10,13,16	ND	ND	ND	ND	ND	ND	0.0024	0.0022
C22:4 n3	ND	0.126	ND	ND	0.001	ND	0.0027	0.0027
C22:5 n3	0.179	0.172	0.152	0.0012	0.001	0.001	0.0093	0.0083
C22:6 n3 DHAC 22:6 all cis-4,7,10,13,16,19 DHA	ND	ND	0.639	ND	ND	0.004	0.0058	0.0049
C23:0	0.099	0.077	0.048	0.0007	0.001	0.0003	0.0012	0.0006
C24:0	0.348	0.314	0.511	0.0023	0.002	0.003	0.0140	0.0035
C24:1 c15	2.009	1.961	2.761	0.0135	0.013	0.018	0.0054	0.0050

ALA = alpha-linolenic acid; AOAC = Association of Official Analytical Chemists; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LOQ = limit of quantitation; ND = not detected; N/A = not available.

*At least 1 batch used in average was below the LOQ. In this case, LOQ was utilized to calculate average (LOQ = 0.01 g/100 g).

A - Presented as the average of 3 batches and includes data provided from commercial chicken products obtained from local marketplace.

Lastly, the approximate vitamin, mineral, and metal content in cultured chicken cells was determined. These data are presented alongside values determined from the analysis of ground chicken, chicken fat, and

chicken breast, as well as the values published by the USDA for raw chicken breast. Analyses were conducted using in-house validated methods or *via* validated methods at a third-party accredited laboratory (see Table 5.4-4).

Table 5.4-4 Analytical Method for Vitamin and Mineral Analyses

Parameter	Method
Vitamins	
Vitamin E (mg/100 g)	UNI EN 12822:204
Vitamin D_3 (µg/kg)	In-house MP 1570 rev 3 2021
Vitamin C (mg/100 g)	In-house MP 2174 rev 3 2019
Folates (µg/100 g)	In-house MP 2346 rev 3 2021 (based on AOAC 2011.06)
Minerals	ICP-MS

AOAC = Association of Official Analytical Collaboration; ICP-MS = inductively coupled plasma–mass spectrometry.

As shown in Table 5.4-5, vitamin, mineral, and metal content of cultured chicken cells were either comparable to or lower than that of conventional chicken products. Of note is the reduction of sodium content in cultured chicken cells as compared to conventional chicken products, which can contribute to a reduction in sodium intake when substituted for conventional chicken. The only metal that is increased in cultured chicken cells is tin at 2.53 ppm. However, this level of tin is far below that of dietary background intakes and is at a level that is of no concern (JECFA, 1989).

Table 5.4-5 Comparison of Vitamin, Mineral, and Metal Content for Cultured Chicken Fibroblasts and Conventional Chicken Breast Products

Parameter	Cultured Chicken Cells			Store Bought		
	Batch 1	Batch 2	Batch 3	Ground Chicken Sample[a]	Chicken Breast Sample[a]	Chicken Breast (USDA ARS, 2019a)
Vitamins						
Vitamin E (mg/100 g)	<0.02	<0.02	<0.02	<1	<1	0.56
Vitamin D_3 (µg/kg)	ND	ND	ND	1.09*[b]	1.58*[b]	1 IU (D2+D3)
Vitamin C (mg/100 g)	<1	<1	<1	1.91*	2.7*	0
Folates (µg/100 g)	78.6±19.1	40±12	37±11	138[b]	76[b]	9
Minerals						
Silver (mg/kg)	<0.05	<0.05	<0.05	<0.01	<0.01	N/A
Aluminum (mg/kg)	<1	<0.1	<1	<1	<1	N/A
Boron (mg/kg)	<2	<2	<2	2*	<2	N/A
Barium (mg/kg)	<0.10	<0.10	<0.50	<0.5	<0.5	N/A
Beryllium (mg/kg)	<0.05	<0.05	<0.05	<0.05	<0.05	N/A
Calcium (mg/kg)	9.0	22.58	11.90	52.17	51.20	50

Table 5.4-5 Comparison of Vitamin, Mineral, and Metal Content for Cultured Chicken Fibroblasts and Conventional Chicken Breast Products

Parameter	Cultured Chicken Cells			Store Bought		
	Batch 1	Batch 2	Batch 3	Ground Chicken Sample[a]	Chicken Breast Sample[a]	Chicken Breast (USDA ARS, 2019a)
Cobalt (mg/kg)	<0.050	<0.050	<0.010	<0.01	<0.01	N/A
Chromium (mg/kg)	<0.050	<0.050	<0.040	0.12	0.04*	N/A
Copper (mg/kg)	0.34	0.38	0.48	0.34	0.24	0.37
Iron (mg/kg)	3.35	4.22	1.96	5.49	4.07	3.7
Lithium (mg/kg)[c]	<0.05	<0.05	<0.05	<0.03	<0.03	N/A
Manganese (mg/kg)	0.07	0.08	0.08	0.13	0.12	0.11
Molybdenum (mg/kg)	<0.01	<0.05	<0.01	0.03	0.03	N/A
Nickel (mg/kg)	<0.05	<0.05	<0.05	0.05	0.06*	N/A
Phosphorus (mg/kg)	684	516	469	1,848	2,176	2,130
Antimony (mg/kg)	<0.01	<0.050	<0.010	<0.01	<0.01	N/A
Selenium (mg/kg)	<0.05	<0.05	<0.05	0.32	0.30	0.23
Strontium (mg/kg)	0.24	0.41	<0.20	0.29	0.20*	N/A
Tin (mg/kg)	0.60	1.02	2.53	<0.1	<0.1	N/A
Titanium (mg/kg)	<0.10	<0.10	0.40	1.25	1.70	N/A
Vanadium (mg/kg)	<0.10	<0.10	<0.01	<0.01	<0.01	N/A
Zinc (mg/kg)	3.17	2.60	0.98	9.04	6.75	6.8
Potassium (mg/kg)	534	384	528	3,428	3,953	3,340
Magnesium (mg/kg)	47	38	43	288.00	360.33	280
Sodium (mg/kg)	835	905	773	2,386	1,634	450
Silicon (mg/kg)[c]	35	27	27	7.09	8.81	N/A

AOAC = Association of Official Analytical Collaboration; EN = European Standard; ICP = inductively coupled plasma; IU = international units; LOQ = limit of quantitation; N/A = not available; ND = not determined.

* At least 1 batch measured was below the LOQ. In this case, LOQ was utilized to calculate average.

[a] Presented as the average of 3 batches and includes data provided from commercial chicken products obtained from local marketplace in Israel.

[b] N=1 for vitamin B9 as total folates.

[c] Considered a potential media residue, therefore Store-Bought Comparators tested individually with a separate in-house ICP method with lower LOQ, based on AOAC 2011.14.

5.5 Microbial Contaminants and Environmental Impurity Analysis

Believer Meats has conducted microbial and environmental testing per its Food Safety Plan (see Section 4.2). The following sections include results from this testing, demonstrating that Believer Meats'

cultured chicken cells are free of microbial and environmental impurities that may originate from the media. In some cases, results have been omitted for brevity; however, these data are available upon request.

5.5.1 Microbial Contamination

Due to the necessity of maintaining aseptic processing conditions within the bioreactor, food spoilage organisms and foodborne pathogens will not be present in the biomass at the end of a production run. The adventitious agent testing requirements outlined in Sections 4.3.3 and 4.3.4 ensure that no bacteria, fungi, mycoplasma, or viruses originating from the donor animal, or cell media, used for production of the MWCB will enter the cultured meat production process. Risk mitigation plans for these hazards are therefore applied during the cell-lined development phase and no further testing downstream of the production process is necessary as there are no animal derived sources used during the meat production process. An operational prerequisite plan will be in place to maintain a high hygienic level. In contrast to the production process, which must be conducted under sterile conditions, the harvest and washing steps are not conducted under aseptic conditions, therefore, low-level contamination of the harvested chicken fibroblasts with microorganisms from the environment (*e.g*., air, wash water, personnel) can occur. The harvest step was therefore monitored by testing of the harvested cell material for a selection of common spoilage and foodborne organisms (Table 5.5.1-1). Organisms were selected based on the risks associated with this stage of the production process, therefore testing for animal derived microorganisms (*e.g*., *Campylobacter* sp.) is not included as there is no source of these species during the production process. Testing for viruses that may originate from the environment (*e.g*., Norovirus) also was not considered necessary as there is no unique hazards at this stage of the production process that would either introduce or propagate these viruses in a manner that differs from conventional food processing. Therefore, control of virus contamination is achieved through the company's management of worker hygiene and use of adequate protective clothing.

No microbial food safety concerns were therefore identified in the harvested lots. Believer Meats microbial lot testing requirements are an ongoing process that reflects the availability of historical data that will be collected for various foodborne organisms (See Table 5.5.1-1). At this time microbial specifications have been established for total plate count, yeast and molds, *E. coli* and *Enterobacteriaceae*. Contamination risks for other microorganisms will be mitigated through the use of various quality procedures including environmental surface sampling, personnel hygiene practices (*e.g*., personal protective equipment, donning and doffing) and periodic sampling of worker hands for indicator organisms. Specifications for *Listeria* were not included as the harvesting surfaces are dry sterilized surfaces and not conducive to *Listeria* growth.

Table 5.5.1-1 Results of Analysis for Three Non-Continuous Batches of Cultured Chicken Cells

Specification Parameter	Specification	Method[1]	Batch No.		
			1	2	3
Total plate count (CFU/g)	<5000	SI 885 Part 3	<10	<10	10
Yeast (CFU/g)	<100	SI 885 Part 8	<10	<10	<10
Molds (CFU/g)	<200	SI 885 Part 8	<10	<10	<10
Escherichia coli TBX (CFU/g)	<10	SI 885 Part 12	<10	<10	<10
Salmonella sp. (CFU/25 g)	Negative	ISO 6579	Negative	Negative	Negative
Enterobacteriaceae (CFU/g)	<100	ISO 21528-2	<10	<10	<10
Coliforms (CFU/g)		SI 885 Part 4	<10	<10	<10
Staphylococcus coagulase (CFU/g)	-	SI 885 Part 6	<50	< 50	<50

Table 5.5.1-1 Results of Analysis for Three Non-Continuous Batches of Cultured Chicken Cells

Specification Parameter	Specification	Method[1]	Batch No.		
			1	2	3
Mesophilic sulphite reducing clostridia (CFU/g)	-	SI 885 Part 9	<10	<10	<10
Listeria monocytogenes (per 25 g)	-	ISO 11290-1	Negative	Negative	Negative
Pseudomonas (CFU/g)	-	CCFRA9.1	<5	<5	<5

[1]All tests were under accreditation (ISO17025) of the Israel Laboratory Accreditation Authority. The laboratory operates under established working procedures which correlate to the International Standard ISO/IEC 17025 in those disciplines where accreditation has been granted, as detailed for each site in the scope for he the certification of accreditation. The tests are within the recognized frameworks of the Ministry of Health as published in the registrations.

5.5.2 Mycotoxins and Secondary Metabolites

Ochratoxin A was analyzed using an in-house procedure, based on Association of Official Analytical Collaboration (AOAC) 2004.10. This test is designed to detect ochratoxin A in matrices such as grains, flours, spices, cocoa, coffee, dried fruits, and feed. The test quantification is limited to the range of 1.0 to 30 µg/kg. The sample preparation involves solvent extraction filtration, and then the sample is subjected to immunoaffinity column. The eluent is then extracted and injected into a high-performance liquid chromatography (HPLC) machine equipped with a fluorescence detector. Ochratoxin A is quantified compared to standard calibration curves and a process control sample monitored by Mérieux NutriSciences (MXNS) Quality Assurance (QA).

Aflatoxin was measured using an in-house procedure based on AOAC 994.08. This test is designed to detect total aflatoxins (B1, B2, G1 and G2) in matrices such as grains, cereals, flour, sugar, pasta, baked goods, spices, cocoa, nuts, and feed. The test quantification is limited to the range of 1.0 to 30 µg/kg. The sample preparation involves solvent extraction filtration, and then the sample is subjected to an affinity column. The eluent is then derivative and injected to HPLC equipped with a fluorescence detector. All Aflatoxins are quantified compared to standard calibration curves and a process control sample monitored by MXNS QA.

Multiple batches of cultured chicken cells were analyzed for aflatoxin and ochratoxin A using HPLC. The results for all batches are below the respective limits of quantitation (LOQ) for aflatoxin and ochratoxin A (LOQ <1.0 µg/kg).

5.5.3 Media Residues

The media components used during the production process are highly water soluble and residues of media are removed from the cellular biomass using the sequential wash steps. Many of the culture media components are common nutrients that are converted into the biomass of the cell or consumed during metabolic processes. The only components with the potential to bioaccumulate were limited to minerals. The safety of the media components was first evaluated using risk assessment approaches using theoretical estimates of residues based on the use levels of the components in the media (See Section 7.1.1 and Appendix B). The safety of the vitamins and minerals was based on compositional testing of the biomass relative to conventional chicken or other commonly consumed foods. Believer Meats has developed a Tiered risk approach to the safety evaluation of the media components based on their common use in food and hazard characterization profile. The majority of media components were adequately addressed based on nutritional testing or through the company's risk assessment approach (Section 7.1.1). A number of organic amines were identified in the media, which required compositional testing for verification of their

safe use. It was demonstrated the organic amines were present in the cultured chicken cells at levels below other commonly consumed foods (*e.g*., infant formula, fruits, vegetables, nuts, cereals, fresh meat) and therefore residues of these components were not of safety concern.

5.6 Shelf-life Stability Information

Believer Meats expects cultured chicken shelf-life to be similar to conventional meat products ranging from 3 to 5 days in 4°C refrigeration and 12 months frozen in -18°C. As products are not exposed to fecal material during production, the source of pathogen contamination is mainly environmental. Due to low lipid level, shelf-life stability was primarily evaluated for water activity by the Nordic Committee on Food Analysis, No. 168 (see Table 5.6-1). Analysis was carried out using accelerated Q10 model encompassing 20 days at 25°C room temperature simulating 1 year at temperature of -18°C (Mizrahi, 2011).

Table 5.6-1 Water Activity of Cultured Chicken Fibroblasts Over 20 Days

Parameter	Cultured Chicken Cells [a]	
	T0	20 Days
Water activity	0.982	0.987

T0 = time zero.

6.0 INTENDED USE AND DIETARY INTAKES

Believer Meats' cultured chicken products are intended to be similar to conventional chicken with respect to nutrition, function, and organoleptic qualities. According to USDA, "*meat is defined as the flesh of animals (including fishes and birds) used as food*." The density of cells in animal tissues can range from 100 million cells/gram in adipose tissue to 500 million cells/gram in dense cardiac muscle.

Believer Meats' cultured chicken cells can be co-mingled with various common ingredients that are permitted for food use or have previously been determined to be Generally Recognized as Safe (GRAS) for their intended use (*e.g*., soybean protein isolate) to produce extruded finished food products (*e.g*., chicken breasts) that are similar to whole food products that are conventionally produced using chicken meat. Food products containing Believer Meats' cultured chicken fibroblasts will be marketed in a manner that is fully substitutional to current food products on the market that are produced from chicken meat.

6.1 Dietary Intakes from Intended Food Uses

Believer Meats' cultured chicken cells are intended for use in the production of finished food products that will be fully substitutional to existing whole food products produced from conventional chicken meat. As such, the dietary intakes of Believer Meats' products were estimated using consumption data pertaining to conventional chicken. Zeng *et al*. (2019) evaluated consumption trends in the U.S. for chicken and other meats from 1999 to 2016 (cumulative and non-cumulative) using Day 1 and/or Day 2 consumption data from 9 cycles of the National Health and Nutrition Examination Survey (NHANES 1999-2016). Based on results from NHANES 2015-2016, the mean *per capita* consumption of chicken by adults (20 years and older; n = 5,017) was estimated to be up to 252 g/week (equivalent to 36 g/day). Although the 90th percentile intake was not reported, this value was crudely approximated to be 72 g/day by doubling the estimated mean intake of 36 g/day. In the absence of food consumption data, this approach for the approximation of the 90th percentile consumption is recommended by the FDA in the Guidance for estimating dietary intake of substances in food (U.S. FDA, 2006). In summary, the complete substitution of chicken in the diet with

Believer Meat's products would result in an estimated mean and 90th percentile consumption of 36 and 72 g/day, respectively.