

Supplementary Information

Chemical and Sensory Analyses of Cultivated Pork Fat Tissue as a Flavor Enhancer for Meat Alternatives

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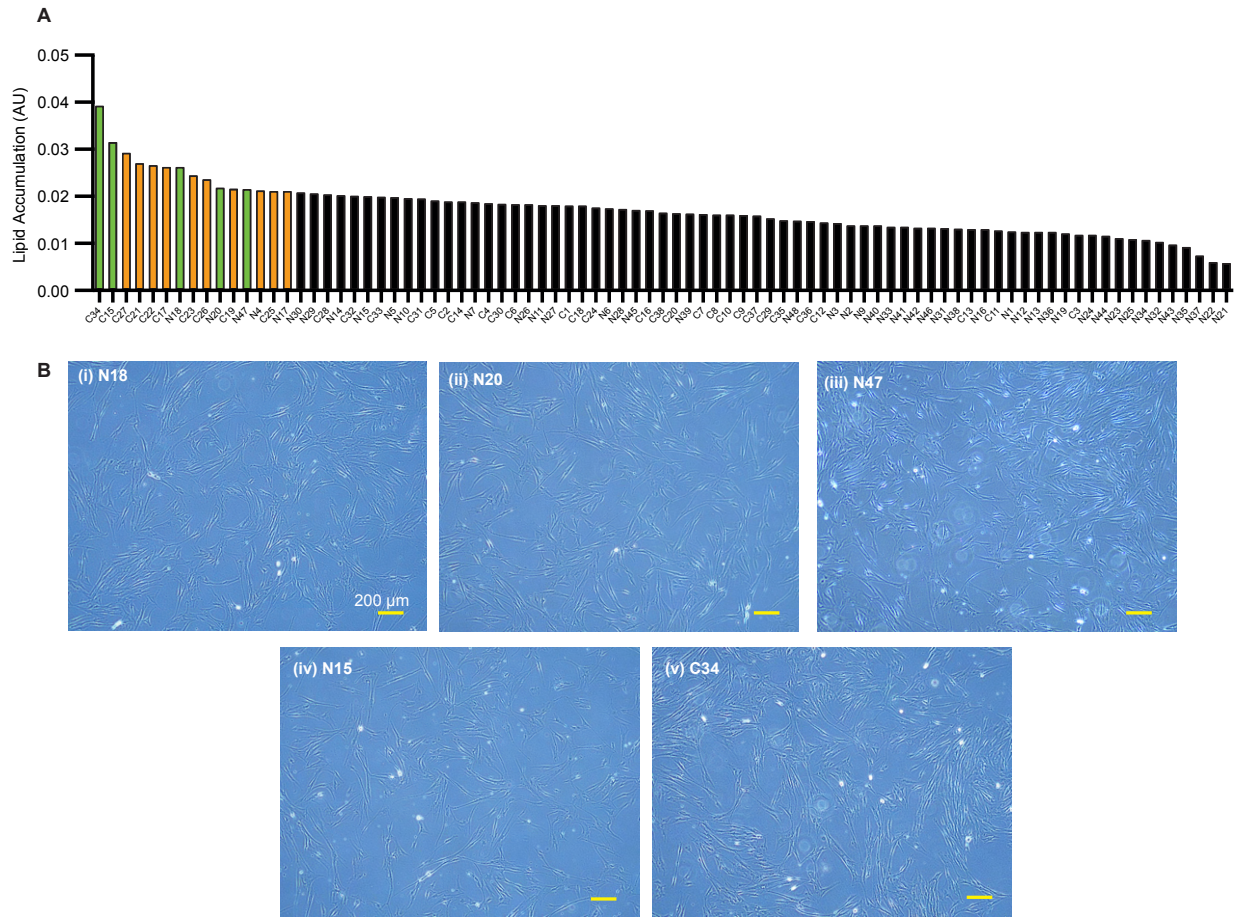
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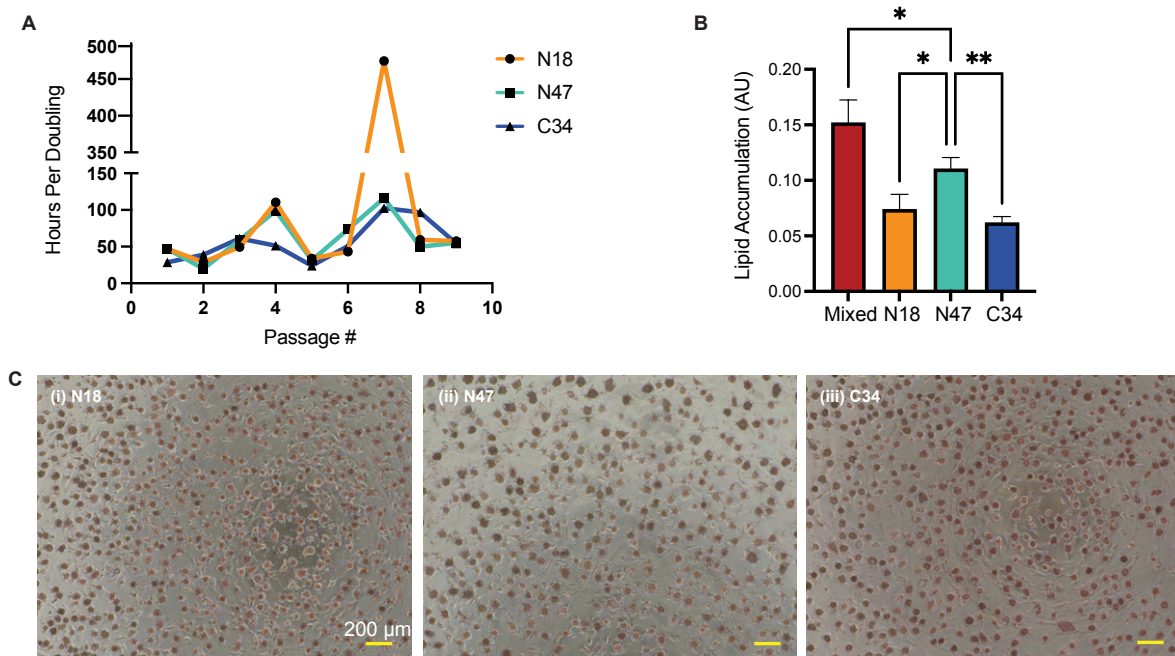
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Supplementary Figure S1: Clonal isolation adipogenesis screening



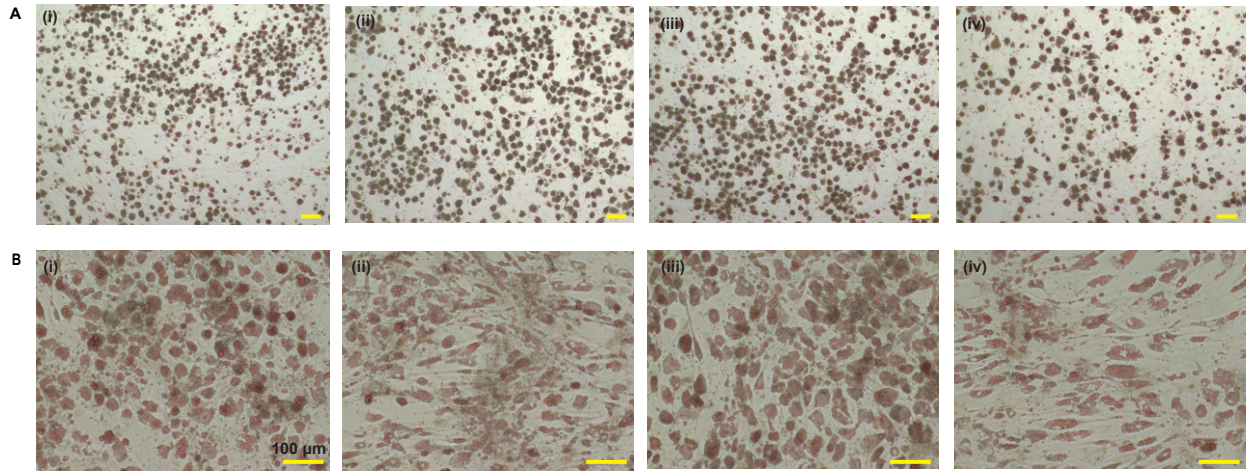
Supplementary Figure S1. Screening of clones isolated from mixed population PDFAT. **(A)** Lipid accumulation screen of normal and conditioned media clones. Clones N1-N48 and C1-C38 were stained with Oil Red O. All clones in vitronectin supplemented media were omitted due to poor proliferation. Clone N8 was omitted due to absorbance value 0. Top 15 clones are highlighted (orange) and clones picked for further screening are highlighted (green). Five clones were selected based on lipid accumulation and previous proliferation screening. **(B)** Morphology of top five clonal populations during proliferation (i) N18 (ii) N20 (iii) N47 (iv) C15 (v) C34 (passage 3). Scale bar represents 200 μm for all images. Scale bar represents 200 μm for all images.

Supplementary Figure S2: Screening of clones N18, N47 and C34



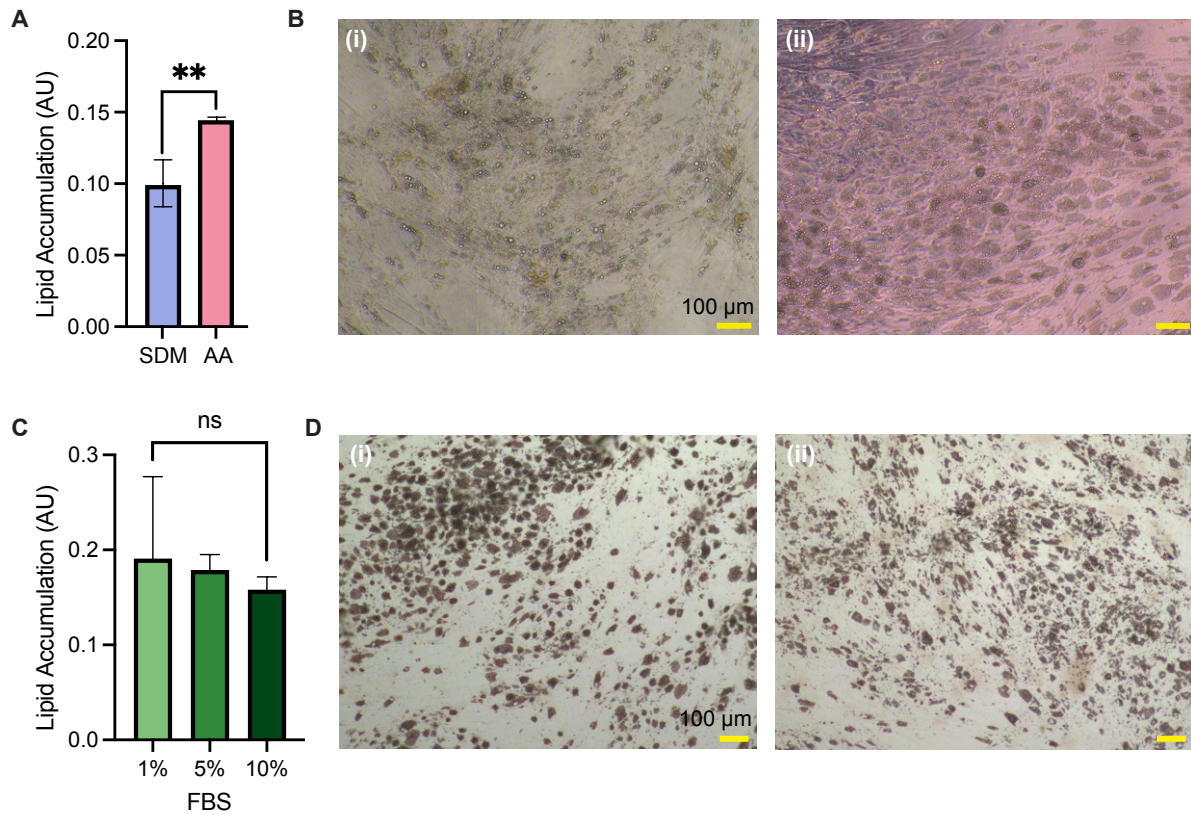
Supplementary Figure S2. Screening of clones N18, N47 and C34. **(A)** Hours per doubling (doubling time) of top three clones N18, N47 and C34 up to passage 9 **(B)** Lipid accumulation screen of mixed population PDFAT and top three clones (P8) determined by proliferation speed up to P6. Screening continued with clone N47. **(C)** Clones (i) N18 (ii) N47 (iii) C34 after six days of adipogenesis and stained with Oil Red O. Scale bar represents 200 μ m for all images.

Supplementary Figure S3: PDFAT lipid morphology



Supplementary Figure S3. Lipid morphology of N47 clones and PDFAT mixed population throughout IBMX, dexamethasone and rosiglitazone media optimization efforts. **(A)** Morphology of N47 cells with various concentrations of IBMX present in the induction media (P12) (A,i) 0 mM (A,ii) 0.1 mM (A,iii) 0.25 mM (A,iv) 0.5 mM. Scale bar represents 100 μm **(B)** Morphology of mixed population PDFAT (B,i) +DEX +ROG (B,ii) -DEX +ROG (B,iii) +DEX -ROG (B,iv) -DEX -ROG in accumulation media (P3). Scale bar represents 100 μm.

Supplementary Figure S4: N47 media optimization



Supplementary Figure S4. Ascorbic acid adipogenic media screen for clone N47. **(A)** Lipid accumulation screen where SDM= standard growth media and AA = SDM supplemented with 113 μ M ascorbic acid as described in Jurek et al. (n= 3). **(B)** N47 (passage 9) after nine days of adipogenesis with (i) SDM and (ii) AA. Scale bar represents 100 μ m (n=4). **(C)** FBS concentration in induction and lipid accumulation medias with clone N47 (passage 23). **(D)** N47 (passage 23) after 10 days of adipogenesis with (i) 1% FBS and (ii) 10% FBS. Scale bar represents 100 μ m (n=3).

Supplementary Table S1: Total compound comparison of conventional and cultivated porcine fat.

(**) tentatively identified with NIST hit.

Compound	Peak Area		Retention Time (mins.)		RI (meas.)	
	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo
1-Hexadecanol	8850120	3221414	19.3	20.4	2369	2503
1-Pentanol	3871048	3163793	20.1	6.9	2464	1258
2-Heptadecanone	2419961	9581884	18.1	18.1	2236	2237
2-Octenal, (E)-	3073749	3885336	8.8	9.3	1392	1434
2,2,4-Trimethyl-1,3-pentenediol diisobutyrate	5508359	15084606	6.6	14.7	1234	1887
2H-Pyran-2-one, tetrahydro-6-nonyl-	2419013	3431779	15.8	21.8	1997	2672
2H-Pyran-2-one, tetrahydro-6-undecyl-	7349251	7576589	12.9	23.8	1721	2901
3-Nonen-2-one	2457239	2917201	14.6	16.7	1877	2082
9-Octadecenoic acid, (E)-	32767970	142004120	22.4	27.1	2741	3066
Acetic acid	27279476	9404231	10.4	10.4	1517	1518
Acetic acid, methyl ester	2326433	5073681	13.3	7.6	1763	1303
Benzaldehyde	1260470	3028198	12.1	10.5	1655	1526
Butanoic acid	3649199	5888981	11.8	11.8	1626	1626
Butyrolactone	4176999	2563600	11.8	11.8	1626	1633
delta-Dodecalactone	3981136	7688113	11.9	19.9	1643	2439
Dibutyl phthalate	7575945	3231474	15.6	22.0	1972	2703
Dimethyl ether	1757173	3870217	14.0	3.1	1820	863
Furan, 2-pentyl-	27107789	4858058	6.6	6.6	1234	1233
Hexadecane	8383065	2130597	11.5	11.4	1600	1599
Hexanal	18069051	6835885	4.7	4.6	1093	1089
Hexanoic acid	37043963	16083840	14.2	14.2	1844	1844
n-Hexadecanoic acid	57742859	145163036	23.9	23.9	2907	2908
Niacinamide	27397941	19010696	24.6	24.6	2972	2971
Nonanal	5291328	11334456	8.9	8.8	1396	1395
Nonanoic acid	7724917	9881773	17.5	17.5	2165	2165
Octadecanoic acid	20376824	48567818	26.6	26.6	3092	3092
Octadecanoic acid, ethyl ester	2988928	1915540	20.1	20.1	2464	2464
Octanal	4156389	5552584	7.4	7.4	1291	1291
Octanoic acid	6628059	45795310	16.4	16.4	2059	2058
Palmitoleic acid	8674152	73204134	24.3	24.3	2943	2944
Pentadecanoic acid	2683743	3395664	22.9	22.9	2800	2801
Pentanal	6715416	3474073	3.5	3.5	886	887
Phthalic acid, isobutyl 4-octyl ester	8077736	3601691	208.0	20.8	2548	2547
Tetradecane	16025271	1949950	8.9	8.9	1400	1399
Tetradecanoic acid	13778200	86431341	22.0	22.0	2695	2696

Supplementary Table S2. Compounds unique to cultivated porcine fat samples

(**) tentatively identified with NIST hit.

Compound	Peak Area	Retention Time (mins.)	RI (meas.)
(E)-9-Octadecenoic acid ethyl ester	7758554	19.9	2439
(Z)-9-octadecen-4-olide	3470941	27.1	3066
1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	2132580	20.3	2485
1-Butanamine, N-butylidene-	4052708	26.4	3068
1-Butene, 3-butoxy-2-methyl-	3501644	5.4	1147
1-Dodecanol	3944123	6.2	1203
1-Tetradecanol	2733637	9.6	1452
1H-Benzocycloheptene, 4,4a,5,6,7,8,9,9a-octahydro-4a-methyl-, trans-	3304041	6.9	1257
2-Decanone	22348937	17.6	2178
2-Fluoroaniline, N-pentyl-	3825081	10.2	1497
2-Heptanone	32461734	19.5	2394
2-Nonanone	21354871	6.0	1189
2-Pentanol, 2,4-dimethyl-	2425799	9.3	1434
2,6-Di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one	3480077	14.7	1887
2(3H)-Furanone, 5-butyldihydro-	2372591	15.8	1997
2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (.+/-.)-	4671823	16.8	2099
3-Octen-2-one	3036754	23.8	2901
4-Oxohex-2-enal	3453521	14.6	1877
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8190230	9.1	1411
6-Undecanol	3480872	23.7	2887
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	2686252	13.3	1764
Acetamide	2594161	13.3	1763
Acetoin	9292528	10.4	1517
Acetophenone	1414578	7.6	1302
Amantadine	2507091	7.4	1290
Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-	3222745	11.7	1621
Benzoic acid	5015234	19.7	2412
Cyclodecane	3736136	11.8	1633
Cyclohexanone, 2-butyl-	2507879	14.0	1820
Dimethyl Sulfoxide	31348131	3.1	864
Ethanone, 1-(3-butyloxiranyl)-	10646196	11.9	1643
Heptadecane	3206681	12.6	1699
Hexadecanoic acid, ethyl ester	3842177	18.3	2258

Methane, isocyanato-	10031276	2.9	850
n-Pentadecanol	7450026	18.5	2280
Pentanoic acid	4933703	13.0	1737
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	3068763	18.8	2306
Phthalic acid, di(2-propylpentyl) ester	15974451	27.5	3175
Propanal, 2-methyl-	11318251	2.3	804
Propanoic acid	10145153	10.7	1537

Supplementary Table S3. Compounds unique to conventional pork fat

(**) tentatively identified with NIST hit.

Compound	Peak Area	Retention Time (mins.)	RI (meas.)
1-Octen-3-ol	3399388	9.6	1452
2-Decenal, (E)-	5990927	12.0	1648
2-Heptenal, (E)-	6872204	7.9	1328
2-Nonenal, (E)-	4614505	10.7	1540
2-Pentadecanone	54228020	16.1	2027
2-Tridecanone	6861489	13.9	1813
2-Undecenal	5719360	13.3	1757
2,4-Decadienal	8272301	13.9	1815
2,4-Imidazolidinedione, 1-methyl-	2932071	22.2	2718
2(3H)-Furanone, 5-acetyldihydro-	3805962	16.5	2063
2(5H)-Furanone, 3-methyl-	3999106	12.9	1721
2H-Pyran-2-one, tetrahydro-6-pentyl-	8581889	17.8	2205
3-Methyl-hexanoic acid	3195677	15.4	1951
3(2H)-Furanone, 4-hydroxy-5-methyl-	3154759	17.0	2118
9-Decenoic acid	28614654	19.0	2332
9(E),11(E)-Conjugated linoleic acid	59967809	28.1	32345
Benzeneacetaldehyde	6169171	12.0	1643
Butanoic acid, 3-methyl-	4842018	12.3	1669
cis-10-Heptadecenoic acid	5391139	25.6	2992
Dimethyl sulfone	2797316	9.0	1409
Dodecanoic acid	42205675	20.3	2483
Ethanol, 2-(2-butoxyethoxy)-	2586821	13.8	1800
Ethanol, 2-(hexyloxy)-	3040865	11.6	1616
Formic acid, octyl ester	2336234	11.0	1562
Glycerin	51668188	18.9	2316
Heptanal	4260583	6.0	1189
Myristoleic acid	5472385	22.4	2741
n-Decanoic acid	111782473	18.5	2272
Oxacyclotridecan-2-one	3389781	20.8	2544
p-Cresol	3327913	16.6	2079
Pentadecane	2704867	10.2	1499
Phenol, 4-(1-methylpropyl)-	2591936	14.3	1852
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	9972895	14.5	1873
Thiophene, 2,3-dihydro-	5896415	19.4	2384

Supplementary Table S4: Triangle test with 54 consumers.

Unalike Triangle Sample	Number of Correct Responses
Pork Belly Fat	20/24
Cultured Pork Fat	21/30
Total	41/54 ^a

^a Critical number of correct responses required to demonstrate statistical significance ($p < 0.05$) in a triangle test is 25/54 ⁴⁰.

Supplementary Table S5: Full triangle test summary

Consumers were presented with one triangle set consisting of 3 samples. Response of “1” indicates successful discrimination of the unalike sample while “0” indicates unsuccessful discrimination. N/A indicates no answer was given.

Consumer	Discrimination
1	1
2	0
3	1
4	1
5	1
6	1
7	0
8	1
9	1
10	1
11	1
12	1
13	0
14	1
15	1
16	1
17	1
18	1
19	1
20	1
21	1
22	0
23	0
24	1
25	0
26	1
27	1
28	1
29	0

30	1
31	1
32	1
33	1
34	1
35	0
36	1
37	0
38	1
39	1
40	0
41	0
42	0
43	1
44	1
45	1
46	1
47	1
48	N/A
49	1
50	1
51	1
52	1
53	1
54	0
55	1

Supplementary Table S6. Consumer demographics as percent of overall study

Age			Gender		
18 to 24	25 to 34	35 to 47	Female	Male	Non-binary
59.3%	35.2%	5.6%	47.3%	47.3%	5.5%

Highest Level of Education					
High School	Some College	Technical School	Bachelor's	Master's	Doctorate
7.3%	32.7%	0.0%	45.5%	7.3%	5.5%

Annual Household Income								
Under \$20,000	\$20,000 - \$39,999	\$40,000 - \$59,999	\$60,000 - \$79,999	\$80,000 - \$99,999	\$100,000 - \$119,999	\$120,000 - \$139,999	\$140,000 - \$159,999	\$160,000 and over
13.5%	11.5%	26.9%	5.8%	5.8%	7.7%	1.9%	3.8%	23.1%

Would you be willing to buy cultivated meat if it has the same price as conventional meat?	Would you be willing to pay 10% more for cultivated meat?*	Would you be willing to buy cultivated meat at a 10% discount?**
Yes: 98.2%	Yes: 81.5%	Yes: 100.0%

* = out of participants who would buy cultivated meat at the same price as conventional meat.

** = out of participants who would **not** buy cultivated meat at the same price as conventional meat.