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Chemical and Genetic Composition Analysis of Organic and Nonorganic Tortilla Chips

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Abstract

The aim of this study was to explore the chemical and genetic differences between organic and nonorganic tortilla chips using GC-MS and PCR. Twenty chip brands were selected: 10 organic and 10 nonorganic. A survey on shopping preferences was created and distributed to compare results of public opinion to experimental data. It yielded 212 responses. All organic chip brands, and one out of ten nonorganic chip brands, tested negative for GMOs. This study concluded that there are minimal chemical differences based on Jaccard similarity indicies and stark genetic differences between organic and nonorganic tortilla chips. In comparing statistical analyses to survey results of public opinion, it was found that the 25.12% price inflation in organic tortilla chips proves to be a barrier that 15% of shoppers are willing to overcome, while the others are content to shop nonorganically or to vary their purchases between categories.

Introduction

Grocery shopping: a mundane, convoluted, yet necessary task in the average US household. Stepping into a grocery store is walking into a stimulating environment, rich with marketing tactics. When considering the cost vs reward comparison that most do while searching the aisles, it can be difficult to feel confident in each purchase decision. When purchasing either organic or nonorganic foods, there are many factors to consider: price comparison, nutrition, diet needs, and personal preferences. To add more complexity to this comparison, the term "organic" is not FDA regulated, and the term "natural" is neither FDA nor USDA regulated. These terms can be subjectively used on food packaging according to each brand.

Both organic and nonorganic farming adhere to certain regulations set by the USDA. For organic farms, the main restrictions lie in what and how often farms spray their crops with pesticides, the use of genetically modified organisms (GMOs), and use of irradiation. Pesticides act as endocrine disruptors in the human body and have negative environmental impacts, such as runoff water and effects on surrounding fauna. However, if these pesticides were derived from an organic source, they are allowed to be used on organic farms. For example, in this experiment, pyrethrins were a pesticide of interest. They are derived from *Chrysanthemum cinerariifolium* and target larvae of various insects[21]. Another pesticide of interest was the BT toxin, derived from *Bacillus thuringiensis*. This can be sprayed onto crops or woven into their genomes. It produces a delta-endotoxin to lower the protective pathway in the alkaline stomachs of caterpillar larvae[20]. It will not affect the protective pathway in humans' acidic stomachs. 85% of crops in the U.S. are exposed to the BT toxin[20].

GMOs can be defined as plants, animals, or microorganisms whose genome has been modified by recombinant genes, microencapsulation, macroencapsulation, or cell fusion[17]. Their negative connotation can partially be attributed to an experiment done by Dr. Arpad Pusztai in

1998[22]. Dr. Pusztai exposed groups of rats to potatoes either laced with lectin, containing genetic modifications, or a parent strain as the control. He prematurely concluded and publicly released results that genetically modified potatoes cause health defects before undergoing the peer review process. His results were later invalidated for design, analysis, and distribution flaws[23]. The FDA has proven that genetically modified genes provide benefits such as producing a higher crop yield by making plants more resistant to drought and insects. They are also cited to retain their freshness and color for longer[17].

Organic foods have somewhat of a health halo in the eyes of consumers, which consequently perpetuates the industry[6]. However, 1 in 5 adults who purchase nonorganically are skeptical of the ingredient integrity in organic foods[6]. As the term “natural” has not yet been defined by the government, there are no parameters around which to make regulations. This does not go unnoticed, as 39% of organic shoppers would like to see clarity on the term “natural” while 44% would like to see increased regulations. Within the same population of organic shoppers, 30% make note that government regulations on organic foods are lacking and too vague[6]. This sparked the question, “Are organic brands following convention and staying true to their claims and promises, or do the increased prices contribute to the health halo surrounding the organic food industry?”

The present study explores differences between organic and nonorganic tortilla chips using gas chromatography mass spectrometry (GC-MS) and polymerase chain reaction (PCR). GC-MS was used to analyze the extraction of various chip samples to determine what compounds they contained and, in particular, if they contained any compounds of concern to human health. PCR was used to detect the presence of GMOs. These results were then compared to a survey that addresses shopping preferences to discuss disparities and similarities between each analysis. Tortilla chips were chosen as the subject of this study as they are a popular snack item with abundant organic and nonorganic options in the US market, and their DNA is easily amplified. It is expected that organic and nonorganic tortilla chips have stark chemical and genetic differences.

Methods

There were 20 types of chips purchased, predominantly, from grocery stores in the Kalamazoo and Portage, Michigan region. Each brand is color coded and has a unique abbreviation, as shown in **Table 1**: nonorganic chips are blue and are denoted by _-N, and organic chips are pink and denoted by _-O. Full size bags with no signs of tampering were randomly chosen. An effort was made to find samples that had little to no added flavoring, with the exception of one sample, and were popular choices for consumers. Samples were extracted within one week of purchase and within minutes of opening the bag. To minimize variation between samples and to give somewhat of a standard with which to compare disparities between chips, the organic and non-organic versions of a type of chip from the same manufacturer were purchased. For example, Meijer brand chips (M-N) were purchased as well as their counterpart, True Goodness (TG-O). The other group that was controlled in this way were chips from Tostitos (T-N) and their counterpart, Simply Tostitos (ST-O).

To test alternative hypotheses, three of the chip samples were not unseasoned tortilla chips. Simply Doritos Organic White Cheddar Tortilla Chips (SD-O) were chosen to explore the additional flavor component and to see how similar these chips would be to a similar one with no additional seasoning. Trader Joes Organic Corn Chip Dippers (TJD-O) were chosen as a unit of comparison to Trader Joes Organic White Corn Tortilla Chips (TJW-O) to explore the difference between corn chips and tortilla chips. Fritos (F-N) were chosen as another example of corn chips that served as a unit of comparison between organic and non to TJD-O.

Table 1. This table shows the 20 types of chips purchased, their abbreviations, and the price paid for them.

Chip Brand	Abbreviation	Price Paid
Meijer	M-N	$2.19
True Goodness	TG-O	$3.29
Tostitos	T-N	$4.59
Simply Tostitos	ST-O	$5.29
On the Border	OB-N	$3.78
Late July	LJ-O	$4.99
Garden of Eatin'	GE-O	$3.99
Chi-Chi's	CC-N	$3.79
Trader Joe's White Corn Tortilla Chips	TJW-O	$3.99
Trader Joe's Organic Corn Chip Dippers	TJD-O	$2.49
Donkey Chips Unsalted	D-N	$4.59
Mi Niña	MN-O	$4.99
Santitas	S-N	$2.49
Xochitl	X-O	$6.29
Pueblo Linda	PL-N	$2.29
Clancy's	C-N	$1.95
Simply Nature	SN-O	$3.49
El Matador	M-N	$3.39
Fritos	F-N	$3.50
Simply Doritos Organic White Cheddar Tortilla Chips	SD-O	$4.59
Organic Chip Average Price: $4.34 ± 0.653	Nonorganic Chip Average Price: $3.25 ± 0.566	

Survey

A survey questioning respondents regarding their shopping habits and preferences was advertised from 01/01/2023 to 05/01/2023. Questions were adapted from "Natural and Organic Food Shopper: Incl Impact of COVID-19" by Mintel Group to compare data. An incentive of a $50 VISA was included, which was disbursed to one winner chosen at random after the collection period. An option to receive the present study after their participation was also offered. Flyers were posted in various buildings around Western Michigan University's main campus and were advertised on Facebook, Instagram, and Snapchat. The purpose of this survey was to collect information on how consumers shop and their preferences based on cost, health, and other factors. These data were compared to that collected experimentally to compare general opinion to findings from the lab. Any questions with an "other" option where the respondents were able to specify were further analyzed. Open responses were condensed into certain categories to better quantify opinion. To be subcategorized, responses must have had specific keywords or met certain criteria, which varied per question.

Metabolite Extraction

For multi-residue pesticide analysis and plant metabolite extraction, a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method was used. Chips were randomly chosen from each bag. 10 g of chip sample was homogenized using a mortar and pestle with 3 mL milli-Q water to swell the cells and ensure a smooth mixture formed. This mixture was combined with 15 mL acetonitrile from Fluka Analytical before 250 μL of 0.0541 M caffeine internal standard was added. Caffeine was chosen as the internal standard because it is not present in chips and did not co-elute with any peaks of note during the preliminary testing of the procedure. Buffering salts, 6 g $MgSO_4$ (anhydrous magnesium sulfate) and 1.5 g NaCl (sodium chloride, table salt), were added before vortexing for 1 minute and centrifugation for 20 minutes at 4 °C and 4000rpm. These stabilize the pH of the solution as well as extract water from the sample. The organic layer was then extracted and cleaned by an EMR-Lipid dispersive SPE medium which removes interferences such as additional sugars, lipids, sterols, proteins, and various pigments. This was shaken and centrifuged using the previous method. The supernatant was then extracted and combined with lipid polishing salts (1.6 g $MgSO_4$, 0.4 g NaCl) before being vortexed and centrifuged for 5 minutes in the same conditions. Finally, 1 mL of supernatant was extracted and combined with about 300 mg $MgSO_4$ as an assurance that all water was removed from the sample. It was then vortexed and centrifuged for 5 minutes before being stored in a freezer at -20 °C before analysis.

GC-MS

1 μL of chip extract was injected and analyzed on a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA). They were separated splitless on a HP5MS capillary column with 5% phenyl methyl siloxane (30 m×0.25 mm×0.25 μm). The column temperature was held isothermally at 40 °C for 2 minutes before increasing to 300 °C isothermally for 19 minutes. The solvent delay was 4 minutes. Helium, the carrier gas, had a flow rate of 1 mL/min. Each chip sample was extracted and analyzed in duplicate. The resulting graphs for each chip were overlaid with the control extraction and compared. Peaks that were not present in the control and had a quality match over 40% were recorded as a "1" denoting the presence of a compound in a sample and "0" denoting its absence. The agglomerative hierarchical clustering (AHC) function in XLSTAT was used to create dendrograms using Jaccard similarity coefficients for a cluster analysis through unweighted pair-group method using arithmetic averages (UPGMA) with extracted compounds.

PCR

To extract plant DNA from the corn chips, PCR was performed using the GMO Investigator Kit from BioRad. Before extraction, PSII and GMO primers were made by combining 11 μL in their respective tubes containing 550 μL master mix. The master mix was composed of dNTPs, buffer, and *Taq* DNA polymerase. The chip sample was homogenized using a mortar and pestle with milli-Q water until it was smooth enough to pipet 50 µL into a microcentrifuge tube containing 500 µL of 6% InstaGene matrix. It was then heated on a block for 5 minutes at 95 °C to remove air bubbles before centrifugation for 5 minutes at 4000rpm. 20 µL of each type of primer was added to their respective tubes before adding 20 µL of chip sample. Samples were then ready for amplification. To ensure there was no contamination, a positive and negative DNA control were prepared in each batch of sample. Both samples were Bio-Rad certified. The thermal cycler used was the PTC-100 Programmable Thermal Controller (MJ Research, INC., Watertown, MA, USA). The program conditions were as follows: initial denaturation at 94 °C for 2 minutes; 40 cycles of denaturation at 94 °C for 1 minute, annealing at 59 °C for 1 minute, and elongation at 72 °C for 2 minutes; final elongation of DNA at 72 °C for 10 minutes.

PCR products were analyzed using a 1.5% agarose gel. It was prepared with 0.75 g molecular biology grade agarose by Thermo Scientific in 50 mL 1x Tris Acetic acid EDTA (TAE) with 3 µL of 10,000x SYBR Safe stain. 8 µL of sample was combined with 2 µL of 6x orange gel loading dye before being loaded into the gel. The loading dye consisted of 20 g sucrose, 100 mg Orange G, and brought to a final volume of 50 mL with water. 1.5 µL of Thermo Scientific GeneRuler 1 kb Plus DNA ladder was combined with 3 µL of dye before loading. Voltage was set at a continuous 105 V for 20–30 minutes in 1x TAE buffer.

Ethics Statement

Western Michigan University's Institutional Review Board approval was not needed for the dissemination of the survey. No further permissions were required.

Results and Discussion

Survey

There were 212 responses. Respondents were a mix of ages and genders. Their locations span the Midwest, with the predominant residence being Southwest Michigan. More accurate data representative of the population of shoppers would have been produced with a larger breadth of respondents.

Table 2. Survey questions asked in order, the number of responses, the nature of response, and how often the response was recorded.

Questions	Number of Responses	Response	Response Rate
1. What is your definition of an organic food?	212	Pesticides	36%
		Processing/preservatives	36%
		Chemicals	32%
		GMOs	22%
		Health	8%
2. When shopping, do you prefer organically or conventionally grown foods?	212	Organic	15%
		Conventional	17%
		Vary	42%
		No preference	26%
3. Is there a noticeable price difference between the two choices?	212	Yes	98%
		No	2%
4. If you are willing to pay the increased prices, please explain why.	139	Quality/health	82%
		Values	17%
		Taste	16%
5. If you shop ORGANICALLY, which of the following characteristics are important to your purchase decisions? Please select all that apply.	97	Other	39%
		Regulations on food quality	20%
		Plant based ingredients	53%
		Sustainable product	53%
		Non-GMO ingredients	42%
		Convenient to prepare	24%
		Natural ingredients	70%
		Trusted brand	43%
		Affordability	66%

		Good taste	86%
6. If you shop CONVENTIONALLY, which of the following characteristics are important to your purchase decisions? Please select all that apply.	135	Other	39%
		Regulations on food quality	10%
		Plant based ingredients	30%
		Sustainable product	16%
		Non-GMO ingredients	16%
		Convenient to prepare	40%
		Natural ingredients	39%
		Trusted brand	53%
		Affordability	90%
		Good taste	86%
7. If you VARY your purchases between categories or do not have a preference, which of the following characteristics are important to your purchase decisions? Please select all that apply.	169	Other	40%
		Regulations on food quality	19%
		Plant based ingredients	38%
		Sustainable product	28%
		Non-GMO ingredients	24%
		Convenient to prepare	43%
		Natural ingredients	43%
		Trusted brand	53%
		Affordability	89%
		Good taste	89%
8. The term "organic" is not FDA regulated. With this information, does your preference towards either method of farming change?	212	Yes	14%
		No	60%
		Need more information	26%

Respondents were asked what their personal definition of an organic food was. This was asked to gather general perception of the term organic and how it relates to their shopping habits. Responses were organized into categories: “pesticides,” “processing/preservatives,” “chemicals,” “GMOs,” and “health” at selection rates of 36%, 36%, 32%, 22%, and 8% respectively. While the first three categories could have been condensed into one, the distinction resided in the connotation given by the respondent. When someone cited pesticides and lack-there-of, they were typically referring to sprays. When preservatives were mentioned, it was typically paired with heavy processing post-harvest. While people tend to give chemicals in and on food a negative connotation, it is important to note that everything consumed is a chemical in one form or another.

They were then asked about their shopping habits. 15% denoted that they shop solely organically, 17% that they shop solely conventionally (nonorganically), and the remaining 68% vary their purchases or do not have a preference.

As expected, when asked if there was a noticeable price difference between organic and nonorganic foods, 98% of respondents said “yes.” To further explore the price disparity between organic and nonorganic tortilla chips, the average prices and their standard deviations were calculated based off of the price paid and compared (**Table 1**). The average price for organic tortilla chips was \$4.34 ± 0.653 and that for nonorganic was \$3.25 ± 0.566. This shows that organic tortilla chips are on average 25.12% more expensive than nonorganic tortilla chips. The weight of each bag was not taken into account for the previous calculations. In reality, the price disparity may be greater. As shown in **Figure 1**, organic shoppers cite affordability as a concern 24% less often than nonorganic shoppers and those that vary their purchases. Clarification was requested on why, if applicable, they are willing to pay increased prices.

Of the 139 responses, 95 were considered. Only those with justification for their organic purchase habits were analyzed. The keywords and topics within these justifications as well as their response frequency were as follows: Quality/health, 82%, Values, 17%, Taste, 16%. “Quality/health” included concerns for additional preservatives or pesticides used in the farming process, as well as personal freshness standards and diet restrictions. “Values” included preference on source, such as buying local produce versus that which were transported transcontinentally, as well as brand loyalty and keywords on packaging such as “organic” and “natural.”

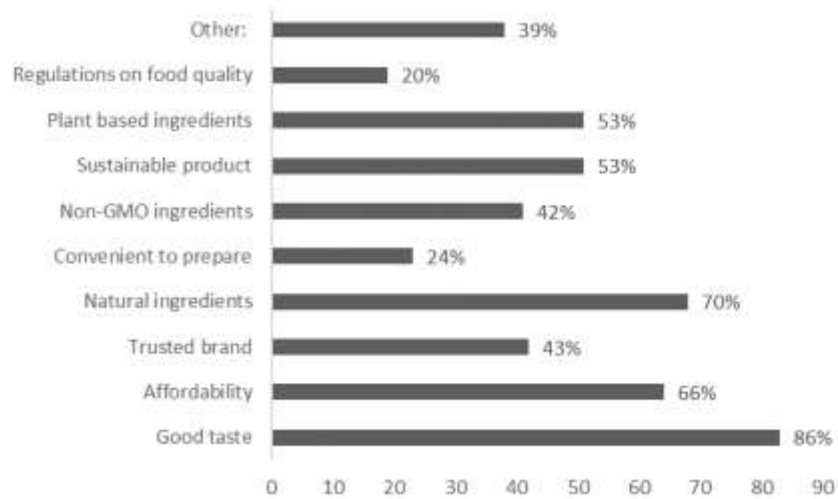

Figure 1. The distribution of 97 responses to the question "If you shop ORGANICALLY, which of the following characteristics are important to your purchase decisions? Please select all that apply."

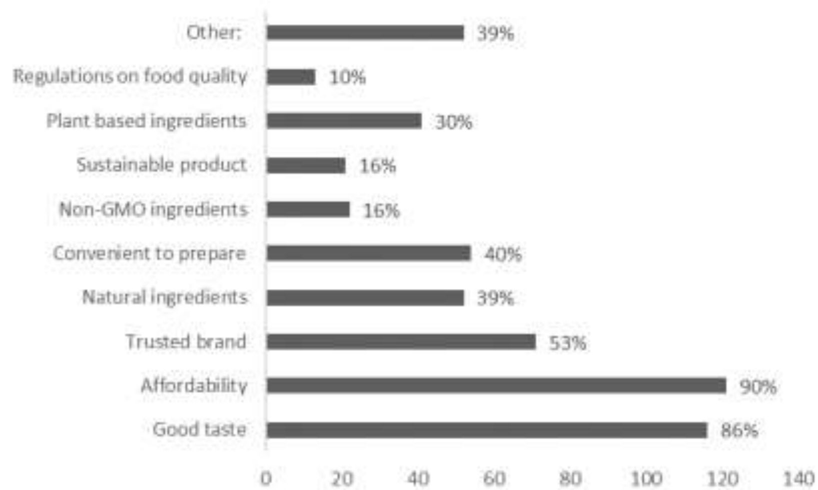

Figure 2. The distribution of 135 responses to the question "If you shop CONVENTIONALLY, which of the following characteristics are important to your purchase decisions? Please select all that apply."

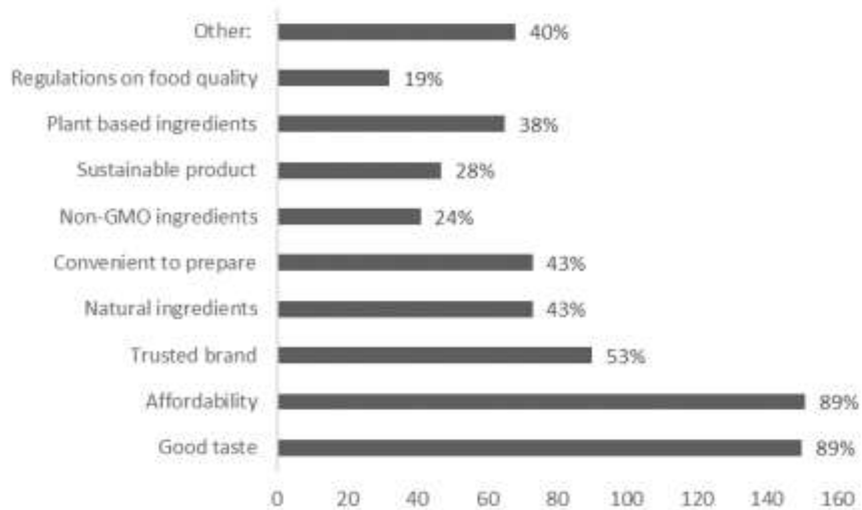

Figure 3. The distribution of 169 responses to the question "If you vary your purchases between categories or do not have a preference, which of the following characteristics are important to your purchase decisions? Please select all that apply."

Questions five through seven were conducted using check-all-that-apply (CATA) methodology **(Fig. 1-3)**. The number of responses for each question did not align with the distribution of shopping preferences indicated in question two. This shows that while respondents were intended to answer one of the questions, some answered more often. In comparing each category of shopper, the choices that were selected most often were "good taste," "affordability," and "trusted brand" at averages of 87%, 82%, and 50% respectively. The popularity of these categories fit the expectation, as shoppers tend to purchase what they've proven to fit their needs and wants. 86% of organic shoppers from question 5 selected "taste" as a concern, whereas only 16% cited taste when justifying their purchase decisions in question 4. This suggests that while taste has importance, they do not initially cite it as justification until the option is presented. Instead, these concerns veer to ingredient type. On average, organic shoppers selected "plant based ingredients," "non-GMO ingredients," and "natural ingredients" 23% more often than the other categories of shoppers. Following suit, they selected "convenient to prepare" 18% less often than the others. This lends to the notion that whole foods and recipes tend to be healthier than packaged foods and premade items. Ways to avoid the latter include shopping the perimeters of a grocery store and reading ingredient labels. The perimeter is where many of the whole foods are, and the shopper is more likely to avoid highly processed foods. By reading ingredient labels, the shopper can compare various products based on how "clean" they are. A "clean" product is defined as something with a short and recognizable ingredient list. Comparing the products used in this experiment shows that organic chips have about 4.9 ingredients, while nonorganic chips have about 6.6 ingredients. These were calculated excluding additional flavor ingredients added to sample SD-O. This shows that organic foods typically have shorter ingredient lists which indicate that they are less likely to be as processed. In comparing the results from **Figure 3** to Figure 6 in Mintel Group's "Natural and Organic Food Shopper: Incl Impact of COVID-19", there are clear parallels between shoppers that vary their purchases (**Appendix A**). The categories, "good taste,", "affordability," and "trusted brand" were again selected most frequently. The only variance of note between the studies is that respondents in the present study selected "plant-based ingredients" about twice as often as the 1,893 respondents in the model study.

Statistical Analysis of GC-MS Chip Composition

There were 168 compounds extracted from 20 samples of chips (**Table 3**). The chemicals were organized into those from the production process, those that may be of concern to human health, and those that may have beneficial effects. All identifications were compared to a standard database. While accuracy would have been improved by comparing each compound to an authentic standard, we did not have access to or the budget for all obtained. The mass spectral library used for identification has not been updated since 2008; however, since all peaks matched the database, we do not think any major components have gone unidentified in this study.

Table 3. This table lists plant metabolite and pesticide residue analysis by GC-MS results in increasing retention time order.

Flavor and Production Compounds	
2,2,3,4-Tetramethylpentane	2-Isopropyl-5-methyl-1-heptanol
Methyl pyrazine	1,1-Diphenyl-ethylene
Ethyl pyrazine	2,3,5,6-Tetrafluoroanisole
2-Furanmethanol	4-Methyl-2,5-dimethoxybenzaldehyde
6-Undecanone	3-(2-Pentenyl)1,2,3, cyclopentanetrione
2-Methyl phenol	Hexadecane
2,2,7,7-Tetramethyloctane	m-Tert-butyl-phenol
2,5-Hexanedione	2-Acetyl-3-ethylpyrazine
Butyrolactone	2,6-Diaminopurine
2,5,6-Trimethyloctane	Thiazolo(3,2-a)pyridinium, 8-hydrxy-2,5-dimethyl hydroxide, inner salt
2,3-Dimethyl pyrazine	3,7,7,Trimethyl-1-3-oxo-but-1-enyl-2-oxa-bicyclo(3.2.0)hept-3-en-6-one
5-Methylthiazole	2,4,6-Trimethyl-1,3-phenylenediamine
Isohexyl 2-propyl ester sulfurous acid	2,3,4,6-Tetramethylphenol
7-Octadecanone	p-Tert-butylphenol
Octylsilane	3,4-Dihydro2H-1-benzothiopyran
Phenol	6-Methyl-2-heptanone
2-(2-ethoxyethoxy)-ethanol	5-Hydroxy-p-t-butylphenol ester pentanoic acid
Hexanoic acid	4-Tert-butylphenyl acetate
2-(2-Ethoxyethoxy)ethanol	Tetradecanoic acid
N-4-Methyl-3-pentenyl-pyrrolidine	Ferulic acid
2,4-Heptadienal	n-Butyl-benzenesulfonamide
1-(2-Methoxypropoxy)-2-propanol	p-Diethylaminoacetophenone
Benzyl alcohol	Methyl ester 1h-indole-3-acetic acid

Benzeneacetaldehyde	Pentadecanoic acid
2-Hydroxybenzaldehyde	Hexadecenoic acid
Methyl-cyclohexane	Heptadecanoic acid
1,1,4-Trimethyl-3-pyrazalone	7-Pentadecyne
1,2-Benzisothiazole	Eicosane
2-Acetyl-1,4,5,6-tetrahydropyridine	1,1-Dioxide1,2-benzisothiazole,3-(hexahydro-1H-azepin-1-yl)
Nonanol	6-Octadecenoic acid
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	9,12,-Octadecadienoic acid
2-Decanol pentafluoropropionate	Oleic acid
Octanoic acid	9-Butyl-1,2,3,4-tetrahydroanthracene
Alitame	1-Butyl-4-nitro-2-propyl-N-(2-hydroxyethyl)-imidazole-5-carboxamide
2,3-dihydrobenzofuran	Aureonone
4-fluorobenzyl alcohol	Pentacosane
1-dodecanol	2-Octylcyclopropaneoctanal
2-Methyl-3-phenyl-propanal	1-(1-Methylene-2-propenyl)-cyclopentanol
Tridecanoic acid	E-9-methyl-8-tridecen-2-ol acetate
Nonanoic acid	1-Bromo-11-iodoundecane
Undecyl pentafluoropropionate	Benzenamine
1-(2-Aminophenyl)ethanone	Octadecanamide
1-(6-Methyl-3-pyridinyl)ethanone	i-Propyl 11-octadecenoate
2,4-Decadienal	2,5-Bis(1,1-dimethylethyl)-1,4-benzenediol
2-Methoxy-4-vinylphenol	2-Amino-5-cyano-6-ethyl-4-(3-pyridinyl), methyl ester 4H-pyran-3-carboxylic acid
4-Hydroxy-3-methylacetophenone	2,4-Bis(1-methyl-1-phenylethyl)phenol
7-Methyl-1H-indazole	Hex-2-yn-4-yl isohexyl ester phthalic acid
2-Butoxy-1-methyl-2-oxoethyl ester butanoic acid	3-Methylheneicosane
n-Decanoic acid	2-Methyl-4,5-diphenyl-4,5-dihydrooxazole
2-Dodecenal	Cinnamyl cinnamate

2-Undecenal	2-Amino-4,4,6,6-tetramethyl-4,6-dihydro-thieno(2,3-c)furan-3-carbonitrile
4-Hydroxybenzaldehyde	Squalene
5-Octadecene	Astaxanthin
Vanillin	1-Chloroheptacosane
3-Hydroxy-4-methoxybenzaldehyde	4-Hydroxyphenyl pyrrolidinyl thione
Bicyclo(4.2.0)octa-1,3,5-triene-7-carboxylic acid	2H-1-benzopyran-6-ol
4-(1-Methylethyl)benzamide	Tetratetracontane
Caryophyllene	N-(a-methylbenzyl)-4-nitrobenzenesulfonamide
N-methyl-2-benzoxazolamine	Hexacosane
1-Benzosuberone	Ergost-7-en-3-ol
Methylcyclooctane	4-Methyl-2-trimethylsiloxy-acetophenone
3-Methyl-1,1-biphenyl	Campesterol
2,5-Bis(1,1-dimethylethyl)-phenol	2,4-Dimethyl-benzo(h)quinoline
1-(4-Hydroxy-3-methoxyphenyl)-ethanone	Sigmasterol
3,5-Bis(1,1-dimethylethylphenol	Diethyl bis(trimethylsilyl) ester silicic acid
Dodecanoic acid	2-Bromo-4,5-dimethoxycinnamic acid
Heptafluorobutanoic acid, heptadecyl ester	Beta sitosterol
Dihydro-5-phenyl-2(3H)-furanone	Gamma sitosterol
Concerning Compounds	
2-(2-Tolyloxycarbonylamino)ethyl ester carbamic acid	Diethyl phthalate
1-Naphthalenol	3-Eicosene
n-Methyl-n-nitroso-2-propanamine	Naphthalene component
1-Methyl-1-phynelhydrazine	4-Nitro-2-diphenylphosphinophenol
Reduced methadone	4-(4-Ethylcyclohexyl)-1-pentylcyclohexane
n-Cyclohexylformamide	Piperonyl butoxide
5-(4-Bromophenyl)-1,3,4-oxadiazol-2-amine	1,2-Benzenedicarboxylic acid, diisooctyl ester
Oxalic acid	2-Ethylacridine

Tetradecane	1,2-Benzisothiazol-3-amine
	5-Methyl-2-phenylindolizine
Beneficial Compounds	
Allopurinol	Vitamin E
1-adamantanecarboxamide	Stigmasta-4,6,22-trien-3alpha-ol
2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	Ergosta-5,22-dien-3-ol
Gamma/beta tocopherol	5,6-dihydroergosterol
DL-alpha-tocopherol	24-methyl-5-cholestene-3-ol
	Stigmasterol

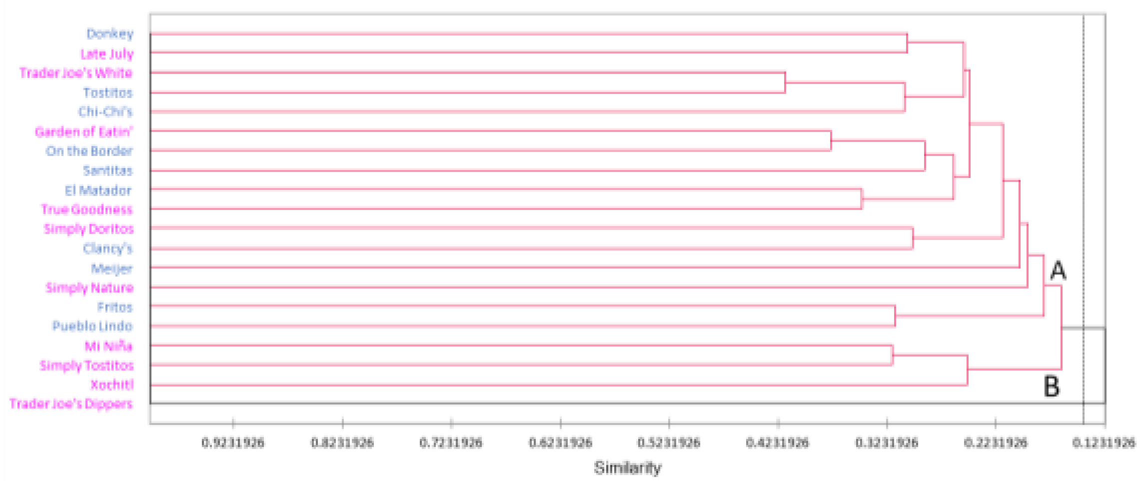

Figure 4. This dendrogram shows the relationships amongst chip samples using Jaccard similarity coefficients for 138 flavor and production compounds released from the production process. There are two main groups labeled A and B.

Compounds grouped into flavor and production include fatty acids from the chip itself or from the frying oils, those associated with color, shelf-life, seasoning color and viscosity, among other properties. In comparing each sample under these parameters, only two clusters were produced, with 19 samples in Group A and 1 in Group B **(Fig. 4)**. The similarity cutoff was at 14.3%. Group A had a grouping interval of 0.179 to 0.417. The samples were largely disbursed throughout these two subgroups, shown by the color coding. This means that while there is some level of similarity between these samples, there is not enough between each of the organic chips or between each of the nonorganic chips for them to segregate based on type, which directly rejects the hypothesis that organic and nonorganic tortilla chips are chemically different as a whole.

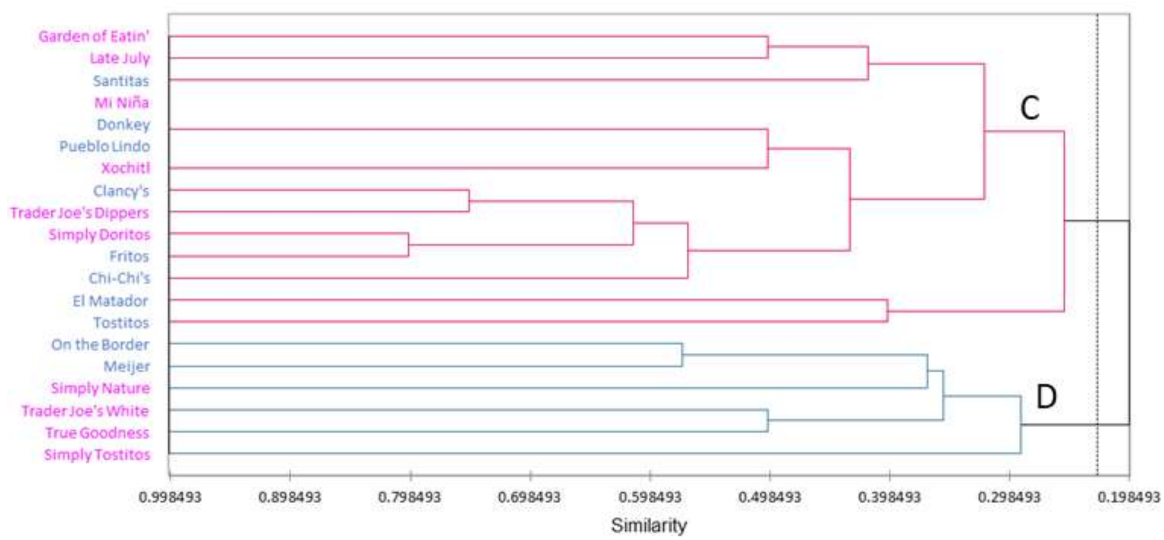

Figure 5. This dendrogram shows the relationships among chip samples using Jaccard similarity coefficients for 19 compounds of concern. There are two main groups, labeled C and D.

Compounds were deemed "concerning" based off potential health deficits including but not limited to pesticides and pesticide synergists, carcinogenic compounds, those found in packaging, and machinery lubricants. Samples were grouped into two, with the similarity cutoff at 26.6%. Group C had a grouping interval of 0.253 to 0.800. Group D's grouping interval was from 0.289 to 0.571. All nonorganic chips were in Group C besides samples M-N and OB-N. Even with these chemical constituents, there does not appear to be a clear difference between these organic and nonorganic samples.

Of the 19 compounds that were deemed particularly concerning, the following were chosen as exemplars for quantitation. Piperonyl butoxide (PBO) is a pesticide synergist that was found in sample M-N. PBO has been reported to form a metabolite-inhibitory complex with P450 enzymes in insects. Inhibition of the cytochrome P450 detoxification system will make the insect more susceptible to the harmful effects of the pesticide with which PBO was paired[18]. While no pesticides were extracted from sample M-N, detecting PBO denotes that there were once pyrethrins or pyrethroids sprayed on the corn used to make the chip. Another compound of concern is naphthalene. It was present in both chips, TJD-O and S-N. While naphthalene levels are typically very low, foods exposed to either fire or smoke (typically non-fish items), tend to have higher concentrations[9]. In sample TJD-O, the concentration of naphthalene was 2.35 μg/g, and sample S-N had a concentration of 8.59 μg/g **(Fig. 5).** This compound is highly present in occupational manufacturing facilities, which serves as a potent threat to the workers. It is also a precursor material for phthalate plasticizers. There is insufficient evidence to claim that naphthalene is a human carcinogen, yet, it has been proven to have carcinogenic properties and promote tumor growth in mice[9]. The compound, diisooctyl phthalate, is a plasticizer used with synthetic rubber and vinyl and has been shown to impair the male mouse reproductive system. The FDA allows its use in adhesives that come into contact with food[12]. It was present in samples LJ-O, GE-O, and S-N in the concentrations 21.18 μg/g, 14.50 μg/g, and 22.0 μg/g respectively. Additionally, tetradecane was present in all chip samples. It is an alkane present in machinery lubricants. When ingested in the human body, it causes inflammation and irritation. In mice, it is carcinogenic and tumor promoting[10]. It was found that on average, the organic chips contained 4.01 μg/g while the nonorganic chips contained 1.94 μg/g. After running a double-sided T-test using two sample equal variance at a 95% confidence level, the p-value was calculated to be 0.4385. Because of the high p-value, it is concluded that the concentration of tetradecane in organic vs nonorganic chips is not statistically different. However, after the intensive chip production process, it is of note that each of these compounds were still present.

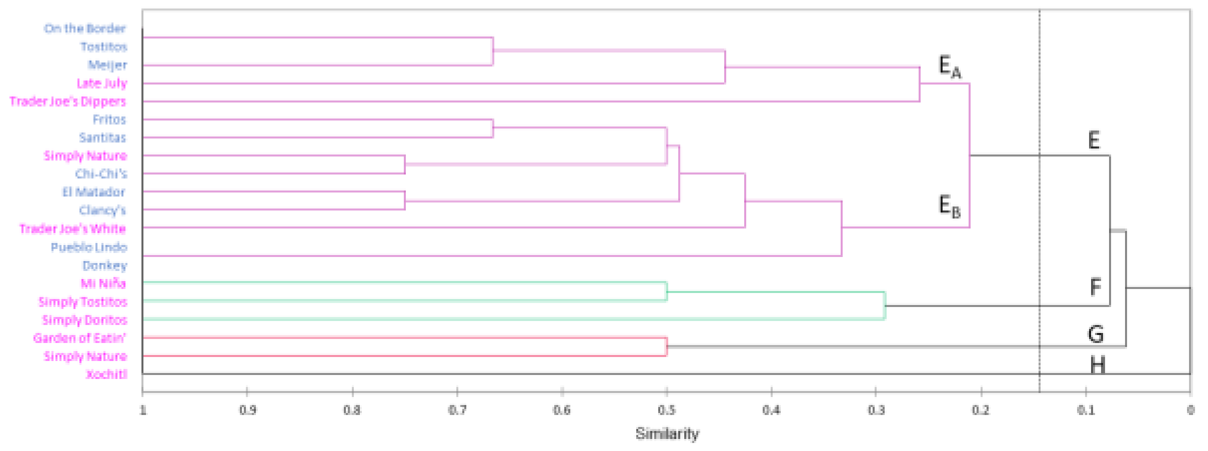

Figure 6. This dendrogram shows the relationships amongst chip samples using Jaccard similarity coefficients for 11 compounds of benefit. There are four main groups, labeled E-H.

Chemicals were deemed "beneficial" based on a variety of factors, such as those that have cholesterol lowering properties and D and E vitamins. The dendrogram fitting these data formed four groups **(Fig. 6)**. The similarity cutoff for this figure is at 14.4%. Group E, the most populated and distinct, had a grouping interval of 0.211 to 0.750. This group houses all the nonorganic chips as well as four of the ten organic ones. Group F had a grouping interval of 0.292 to 0.500 and contained samples MN-O, ST-O, and SD-O. Group G had a grouping interval of 0.062 to 0.500 and contained samples GE-O and SN-O. Group H contained X-O as its solitary sample. Once again, there is not a clear distinction between organic and nonorganic tortilla chips.

Figure 7. This dendrogram shows the relationships amongst chip samples using Jaccard similarity coefficients for all 169 compounds. There are four main groups labeled I-L.

In comparing all compounds of all chip samples, the resulting dendrogram showed 4 groups, with the similarity cutoff at 18.8% **(Fig. 7)**. Group I contained 16 chips with a grouping interval of 0.201 to 0.381. All the nonorganic chips were in this group. There were 4 organic chips in this group: MN-O, LJ-O, GE-O, TJW-O. Each of the following groups contained the remaining organic chips. Group J contained just TG-O with a grouping interval of 0.157 to 0.176. Group K contained chips S-O and X-O with a grouping interval of 0.157 to 0.200. Group L contained just TJD-O which showed the lowest similarity to the other samples. Sample TJD-O showed no similarity to the rest of the population in both **Figures 4** and **7**. This could be attributed to the fact that this sample is a corn chip rather than a tortilla chip. However, the other corn chip, sample F-N, consistently had an average similarity in comparison to the other samples.

Alternative hypothesis testing with samples SD-O, TJD-O, and F-N did not yield results that urged further exploration in the context of this study. However, observations of note include that the added seasoning on SD-O did not seem to largely affect its similarity or lack of such to the other chips when analyzing the combined flavor profiles of each sample. Samples TJD-O and TJW-O proved to be quite different in all categories. While they were in the same group in **Figure 6** they only had a similarity coefficient of 0.212. Samples TJD-O and F-N appeared in the same groups when comparing compounds of concern **(Fig. 5)** and compounds of benefit **(Fig. 6)**, but never appeared in the same subgroup. This is interesting as both types of chip are made from masa instead of heavily processed corn like the other samples. More research must be conducted to determine relationships between organic and nonorganic corn chips, as well as determining the impact, if any, that additional seasoning has when comparing types of tortilla chips.

To account for potential sources of errors, samples M-N and TG-O, both made by Meijer, and T-N and TS-O, both made by Tostitos, were chosen in tandem to control for the manufacturer. However, there are numerous determinate and indeterminate errors that cannot be accounted for in the present experiment. For example, it was not possible to control where the corn was grown, how it was shipped, the conditions in which it was stored, or how long it had been after manufacturing before purchase.

PCR Screening for GMO Chips

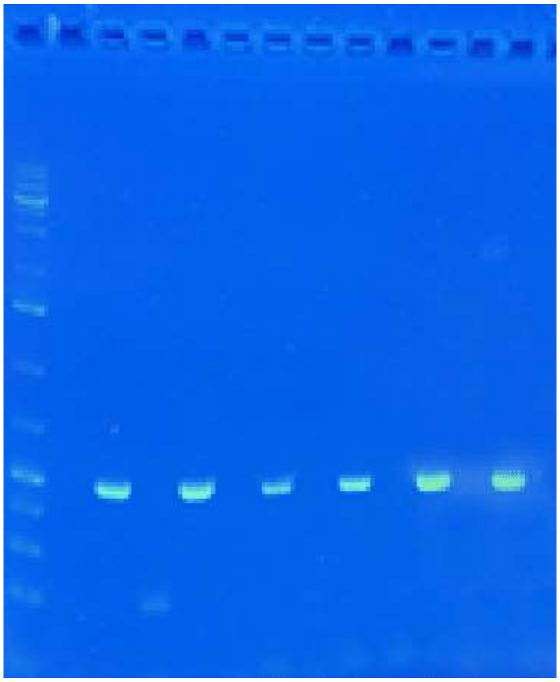

Figure 8. Agarose gel electrophoresis of amplified PCR products of the PSII gene and GMO genes, CaMV 35S and NOS. Lane 1: DNA ladder, lane 2: GMO negative control, lane 3: M-N GMO, lane 4: M-N PSII, lane 5: TG-O GMO, lane 6: TG-O PSII, lane 7: D-N GMO, lane 8: D-N PSII, lane 9: X-O GMO, lane 10: X-O PSII, lane 11: SN-O GMO, lane 12: SN-O PSII

Table 4. Genetic analysis of organic and nonorganic chips by PCR. A "+" indicates that the gene was detected by PCR, while an empty space indicates that the gene was not detected by PCR.

Chip Brand	PSII Gene	GMO Gene
Meijer	+	+
True Goodness	+	
Tostitos	+	+
Simply Tostitos	+	
On the Border	+	+
Late July	+	
Garden of Eatin'	+	
Chi-Chi's	+	+
Trader Joe's White	+	

Trader Joe's Dippers	+	
Donkey	+	
Mi Niña	+	
Santitas	+	+
Xochitl	+	
Pueblo Linda	+	+
Clancy's	+	+
Simply Nature	+	
El Matador	+	+
Fritos	+	+
Doritos Simply	+	

The gene ladder, Thermo Scientific GeneRuler 1 kb Plus DNA ladder, indicates presence of the PSII gene at 455 base pairs (bp) and two GMO DNA sequences: the 35S promoter of the cauliflower mosaic virus (CaMV 35S) at 203 bp, and the terminator of nopaline synthase (NOS) from *Agrobacterium tumefaciens* at 225 bp. This is done by duplex PCR. The two genes are similar in size, so it can be difficult to distinguish which, or if both, were present in a particular sample. Higher resolution separation would have required the use of a polyacrylamide gel, which can be hazardous to use. Nonetheless, the presence of a GMO gene was able to be reliably detected and for the purposes of this project, that distinction was sufficient. **Figure 8** is a gel representative of the population. All samples shown presented negative for GMOs besides sample M-N in lane 3. This was used as this run's positive control, as it had previously been proven positive in comparison with the positive control from the BioRad GMO Investigator Kit. All samples showed the amplified PSII gene. All organic samples were negative for GMOs, while all nonorganic samples were positive except for sample D-N. To confirm, this sample was extracted and run in duplicate. This shows that for those shoppers that prioritize avoiding GMOs but solely shop nonorganically, Donkey chips are the best choice. However, in analyzing price disparity between chips that either lack or contain GMOs, it is evident that in order to consistently eat non-GMO tortilla chips, the consumer must pay more than that for nonorganic chips. The price paid for Donkey chips was $4.59, which falls within the standard deviation of the mean for organic chip price.

Conclusion

This research explored the chemical and genetic differences between organic and nonorganic tortilla chips to compare to a survey on shopping preferences. Results have shown that while organic and nonorganic tortilla chips do not vastly differ in chemical composition, they do have clear genetic disparities. DNA isolation and analysis was done by PCR and proved that all samples of organic chips tested were GMO free, while all but one sample of nonorganic chips contained GMOs. According to the metabolite extraction results and survey responses, organic and nonorganic tortilla chips do not chemically differ as much as the consumer is led to believe.

The results of this study are not intended to be taken at face value, as there are limitations that affect the accuracy of the GC-MS and survey results. The survey would have improved credibility if the number of respondents more accurately represented the population of shoppers in the U.S. Due to the large number of samples, a more comprehensive analysis of all compounds present would be beneficial in comparing types of chips. Additionally, it would be beneficial to use an instrument with a current mass spectral library and compare these compounds to authentic standards to confirm identity before making claims.

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Lee Honors College Research & Creative Activities Scholarship – Fall 2021, Spring 2022

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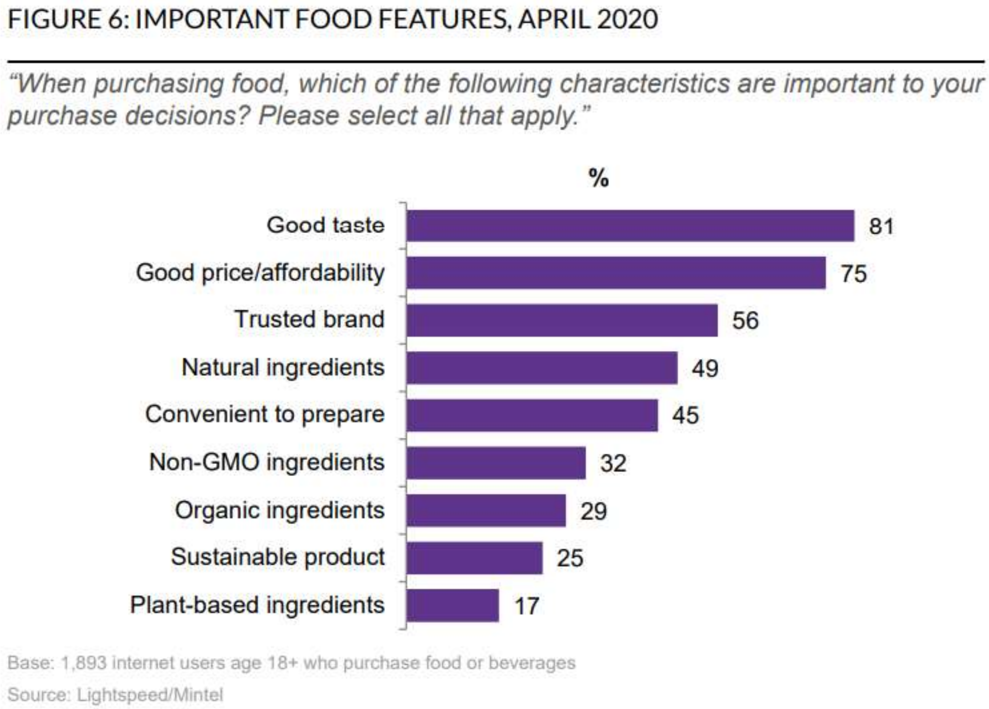
Appendix:



Appendix A. This figure is a chart from Mintel Group's ""Natural and Organic Food Shopper: Incl Impact of COVID-19"