Project 1 Manuscript

Accuracy of Proximate Analysis of Crude Ash Content of Sunflower Seed Powder Using the Muffle Furnace

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Introduction:

Accurate data is extremely important in analytical food science. Unreliable data is of no value to the investigator and wastes valuable lab resources. The instruments in PSFA 416 vary in their degrees of specificity and sensitivity, speed of measurement, technological and chronological age, and required maintenance. The purpose of this investigation is to quantify the reliability of the instruments in PSFA 416 in measuring the compositional and physical properties of sunflower seed powder by official methods of analysis. Of the 5 major components of a food (moisture, ash, fat, protein, and carbohydrate), this experiment looked specifically at the crude ash content. Determining the ash content of foods is important for accurate nutritional labeling of mineral content and raising awareness of possible toxicity, as well as quantifying certain minerals that are important for a healthy diet and including that on the nutrition label. It is also important for determining quality of a food, including taste, appearance, texture, and stability. Just like measuring water, measuring ash content helps with understanding the microbial viability of a food. The level of mineral content found in food indicates the magnitude of microbial stability. Natural foods will tend to be closer to 5% ash content while processed foods contain closer to 10% ash content. When calculating the ash percentage, it can be expressed in dry weight or wet depending on if you're measuring for oxidation or proximate composition. In the method of dry washing, water & volatiles are vaporized leaving most inorganic minerals to be converted to chlorides, phosphates, sulfates, or oxides. There are elements such as Pb, Hg, Se, and Fe which can partially volatized, so if specific element analysis is desired, wet washing is better to use. Ashing in the food industry is especially important to test for specific mineral content in plants, because they have a much higher degree of variability than animal products. It's important to know that ash content on nutritional labels doesn't reflect mineral value, rather it's better that you read the specific mineral amount in the food product.

Methods:

Sample Handling Protocol

Sampling Methods for Wheat Flour (AOAC Method 925.08), modified for Sunflower Seed Flour. Think. Eat. Live. Sunflower Seed Flour was analyzed. Laboratory samples were extracted using a cylindrical, polished, ½", 13mm metal trier with 1/3" circumference slit. Samples were drawn from top right and bottom left corners of the sack and were transferred into glass jars, inverted and rolled 25 times. Samples were stored in dry, sealed, airtight containers at 72 degrees C.

Procedural Steps of the Ash Analysis Using the Muffle Furnace

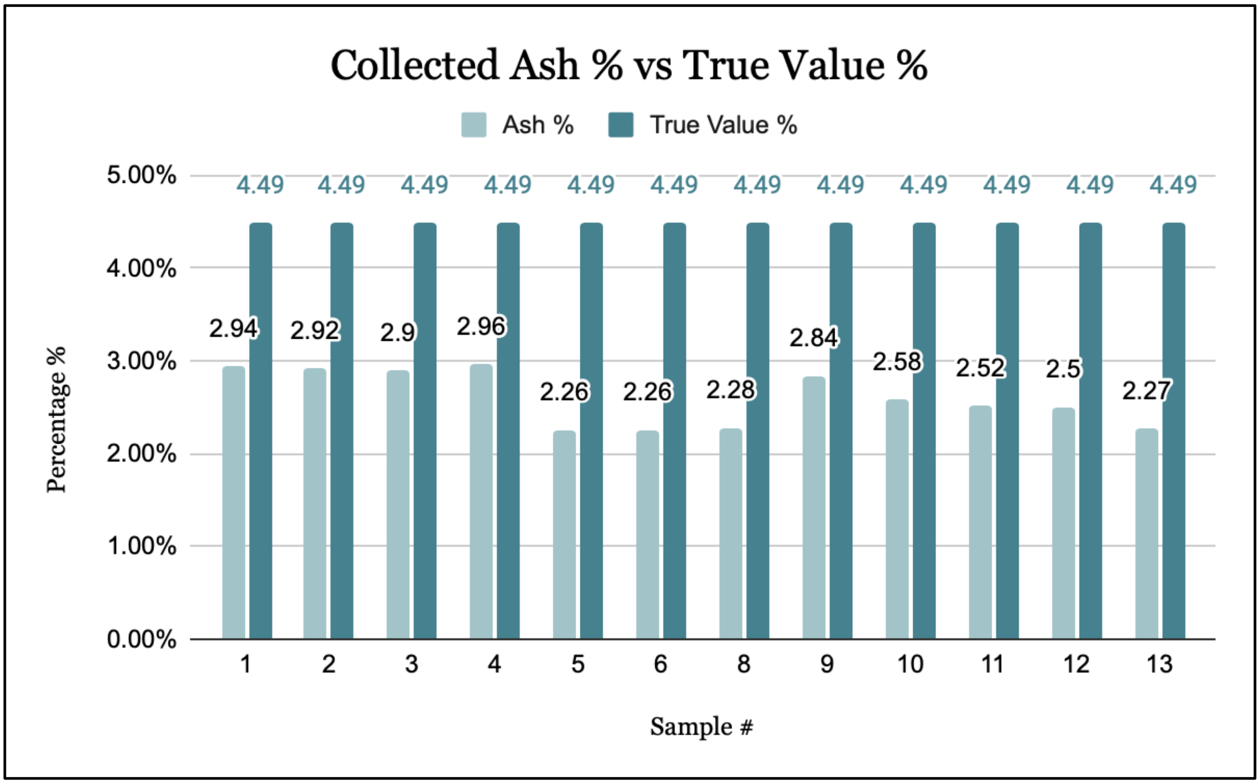
Laboratory sunflower seed powder was brought over to the weighted measuring scale, along with 13 crucibles. The crucibles are made of porcelain in order to withstand the high temperature in the furnace. Thirteen crucibles were used total for analysis of the sunflower seed powder. The crucibles were labeled, one with a "1", two with a "2", counting up to 13. This was done using ferrous iron so it does not burn off in the furnace. Using a leveled scale, crucible "1" was placed on the scale and the weight was recorded. The scale was tared back to 0.00 and 2.0 grams of sample was placed into the empty crucible. This procedure was repeated for samples two through thirteen. The 13 crucibles containing samples were placed with large metal tongs into the muffle furnace. The muffle furnace was used for dry ashing for analysis of ash composition of the sunflower seed powder. Positions in the furnace are labeled with numbers on a diagram to correspond with the crucibles being placed inside. There are different shelves in the furnace and every shelf represents one section. Samples were spread out evenly inside the muffle furnace, corresponding with the labeled diagram. The furnace power was then turned on. The temperature display on the furnace is broken so an external thermometer plugged into it was used. The furnace reached 550 degrees Celsius and was left on for 24 hours. The furnace was turned off. Wearing safety goggles and thick heat protective gloves, samples were taken out using the metal tongs, and then carefully placed into the desiccator for 30 minutes to reach room temperature. The desiccator is a sealable enclosure used for preserving moisture-sensitive items, in this case, ash. It protects chemicals and substances that react with water from humidity. Allowing samples to reach room temperature inside the desiccator preserves any lost sample from volatilization. Each of the crucibles were weighed and recorded. The weight of the ash was found by subtracting the original weight of the crucible alone, by the weight of the crucible with the ash. To determine the percent ash content, the weight of the ash was divided by the original dry weight of 2.0 grams of sunflower seed powder and multiplied by 100.

Formula: (Dry wt/Org wet wt)*100 = % Ash

<u>Results</u>:

Raw data collected from samples one to thirteen consisted of crucible weight (ranged 10.3086 - 43.1615 grams), sample weight (ranged 2.002 - 2.1224 grams), post weight (ranged 10.3686 - 42.949 grams), ash weight (ranged 0.0345 - 0.062 grams), and ash percentage (ranged 1.721385091% - 2.962532675%) (refer to Appendix A). A Q-test was conducted for sample seven (1.72% ash) and was rejected after the Q-value was calculated to be 0.43%, which is greater than 0.36% on Dixon's Critical Value Chart (refer to Appendix B). Of the raw data for ash percentage, the calculations (rounded to the nearest hundredth) were as follows: average (2.60%), standard deviation (0.28%), relative error (42.04%), and coefficient of variation (10.88%) (refer to Appendix C). Ash percentages of each sample (rounded to the nearest hundredth) which ranged from 2.26% to 2.96% were compared to the true value of ash percentage (4.49%) (refer to Table I).

Table I: Collected ash % compared to true value % ash (4.49%) of sunflower seed powder with sample seven being rejected after conducting a Q-test.



<u>Discussion and Conclusion</u>:

In order to measure the accuracy and precision of the data collected for this experiment the raw data collected from samples, numbered one to thirteen, were recorded onto a table.

The table included crucible weight, sample weight, post weight, ash weight, and ash percentage. After the data was recorded, Sample seven had a value of 1.72% for ash percentage, which was questionable compared to the rest of the data. A Q-test was then conducted to determine if the data would be rejected before moving further. A Q- test takes the questionable value subtracted by the next closest value, which is then divided by the spread of all data. The Q-value was determined to be 0.43%. Once the Q-value was solved, it was compared to the 95% Critical Limit column of Dixon's Critical Value chart, which showed a value of 0.36%. Since the Q-value was greater than the value given in the test table, Sample seven of the experiment was then rejected for any further calculations (refer to Table 2). The average ash percentage taken from the samples was calculated to be 2.60% with a standard deviation of 0.28%. Relative error was calculated by subtracting the true value of ash from the ash percentage average (2.60%), then multiplying the absolute value by 100, which resulted in a value 42.04%. By dividing the standard deviation by the average ash percentage, then multiplying by 100, we are then able to calculate the coefficient of variation, which equals 10.88%. These values were used to compare to the true value of ash percentage (Aishwarya and Anisha 2014).

Table 2: Samples to be included in further calculations (average, standard deviation, coefficient of variation, and relative error) with rejected sample highlighted in red.

Sample #	Crucible Weight (g)	Sample Weight (g)	Post Weight (g)	Ash Weight (g)	Ash %
1	10.3086	2.0392	10.3686	0.06	2.942330326
2	11.8876	2.1224	11.9496	0.062	2.921221259
3	11.5072	3.0377	11.5662	0.059	2.895421308
4	12.9375	2.0658	12.9987	0.0612	2.962532675
5	39.5076	2.0081	39.553	0.0454	2.260843538
6	42.9037	2.0056	42.949	0.0453	2.258675708
7	39.2365	2.0042	39.271	0.0345	1.721385091
8	39.6554	2.002	39.701	0.0456	2.277722278
9	25.1372	2.1057	25.1971	0.0599	2.844659733
10	41.8689	2.0292	41.9212	0.0523	2.577370392
11	43.1615	2.0914	43.2142	0.0527	2.519843167
12	40.478	2.0302	40.5288	0.0508	2.50221653
13	39.5117	2.0169	39.5574	0.0457	2.265965887

Outliers may inhibit effective data analysis, therefore conducting a Q-test on a questionable sample that appears to be distant in ash percentage from the rest of the data is beneficial to the experiment. Standard deviation lets us measure the variation of data. A 95% confidence interval (two standard deviations: ± 0.56%) was conducted from the calculated mean. This gives us a range with 95% confidence that the true value lies within range. Since we know the true value (4.49%) is not within the interval of 2.60% ± 0.56%. In order to determine accuracy and precision of our data, relative error and coefficient of variation were calculated. Accuracy measures how close our measured data is to the true value, while precision is the ability to reproduce the same results under unchanged conditions. Coefficient of variation tells us how precise our measurements are. Coefficient of variation is related to standard deviation and must be five percent or below in order to be accurate. Since our coefficient of variation was calculated to be 10.88%, there was no precision within our experiment. Relative error on the other hand, tells us how accurate our measurements are and is directly related to the mean. In order to be considered accurate, data must be five percent or less. With our relative error being 42.04%, our data showed no accuracy. Based on these calculations, there may have been errors somewhere during the experiment.

A few errors may have unintentionally occurred during the experiment, which lead to lack of accuracy or precision. While weighing sample number one, one error occurred. The scale was not tared back to 0.00, leading to a highly inaccurate mass. Another potential error was related to the furnace temperature since the internal thermometer was broken. An external thermometer was used to measure the heat inside the furnace, resulting in a slight inaccurate temperature measurement. Another experimental error occurred while taking out the samples from the furnace. Some of sample seven accidentally fell down before putting it into the desiccator. This led to volatilization of some dry ash from the crucible since it was still hot. Dry ash is more prone to volatilize under high temperature levels (Simons, 2017).

This likely affected the precision of the crucible and ash weight. Having thirteen samples placed among four sections inside the furnace may have led to some errors during the experiment, such as position mix ups. A systematic error occurred due to sample number thirteen being in an air loose container. This was an equipment error which led to inaccurate results. Having some errors along with lack of preparation can impact the samples analysis negatively (*Proximate Composition Analysis*, 2016). These errors may largely affect the experiment reliability.

Conclusion:

Determining the ash content of food is an integral part of proximate analysis for nutrition evaluation. Proximate analysis is the quantitative analysis of macromolecules in food, such as moisture, ash, carbohydrate, protein, and lipid. Inorganic particles that are left behind after food is burned or oxidized are known as ash. The muffle furnace method was used in this experiment to determine the accuracy of crude ash that contains sunflower seed powder. Due to the random errors and equipment errors throughout the experiment, there was a disparity between the ash percentage and the true value percentage. This led to lack of accuracy and reliability. The true value of ash was 4.49%, whereas the average ash percentage of the data values calculated was 2.60% ± 0.56%. Relative error was calculated to be 42.04% with the coefficient of variance being 10.88%. With relative error not being five percent or less, there was no accuracy with this experiment. In addition to no accuracy, there was no precision in this experiment due to the calculated coefficient of variation being greater than five percent. In conclusion, due to the lack of accuracy and precision in our results, the measurements of sunflower seed powder may not be considered reliable. Further research needs to be done in the future to reduce the number of errors in this trial and to achieve better results.

References

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Appendices:

Appendix A: Raw data - Samples one to thirteen for crucible weight, sample weight, post weight, ash weight, and ash percentage. Weight is calculated in grams.

Sample #	Crucible Weight (g)	Sample Weight (g)	Post Weight (g)	Ash Weight (g)	Ash %
1	10.3086	2.0392	10.3686	0.06	2.942330326
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9	25.1372	2.1057	25.1971	0.0599	2.844659733
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12	40.478	2.0302	40.5288	0.0508	2.50221653
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Appendix B: Dixon's Critical Value Chart - Row 13 and column 95% were used to compare calculated Q-value (0.43%) to critical value (0.36%).

Critical values for six Dixon tests for outliers 139

Table 2. Critical values for Dixon-type discordance test N7 of an upper outlier in a normal sample.

n	CL / SL / α	70% / 30% / 0.30	80% / 20% / 0.20	90% / 10% / 0.10	**95% / 5% / 0.05**	98% / 2% / 0.02	**99% / 1% / 0.01**	99.5% / 0.5% / 0.005
3		0.6836	0.7808	0.8850	0.9411	0.9763	0.9881	0.9940
4		0.4704	0.5603	0.6789	0.7651	0.8457	0.8886	0.9201
5		0.3730	0.4508	0.5578	0.6423	0.7291	0.7819	0.8234
6		0.3173	0.3868	0.4840	0.5624	0.6458	0.6987	0.7437
7		0.2811	0.3444	0.4340	0.5077	0.5864	0.6371	0.6809
8		0.2550	0.3138	0.3979	0.4673	0.5432	0.5914	0.6336
9		0.2361	0.2915	0.3704	0.4363	0.5091	0.5554	0.5952
10		0.2208	0.2735	0.3492	0.4122	0.4813	0.5260	0.5658
11		0.2086	0.2586	0.3312	0.3922	0.4591	0.5028	0.5416
12		0.1983	0.2467	0.3170	0.3755	0.4405	0.4831	0.5208
13		0.1898	0.2366	0.3045	0.3615	0.4250	0.4664	0.5034
14		0.1826	0.2280	0.2938	0.3496	0.4118	0.4517	0.4869
15		0.1764	0.2202	0.2848	0.3389	0.3991	0.4385	0.4739
16		0.1707	0.2137	0.2765	0.3293	0.3883	0.4268	0.4614
17		0.1656	0.2077	0.2691	0.3208	0.3792	0.4166	0.4504
18		0.1613	0.2023	0.2626	0.3135	0.3711	0.4081	0.4423
19		0.1572	0.1973	0.2564	0.3068	0.3630	0.4002	0.4333
20		0.1535	0.1929	0.2511	0.3005	0.3562	0.3922	0.4247
21		0.1504	0.1890	0.2460	0.2947	0.3495	0.3854	0.4173
22		0.1474	0.1854	0.2415	0.2895	0.3439	0.3789	0.4109
23		0.1446	0.1820	0.2377	0.2851	0.3384	0.3740	0.4051
24		0.1420	0.1790	0.2337	0.2804	0.3328	0.3674	0.3986
25		0.1397	0.1761	0.2303	0.2763	0.3287	0.3625	0.3935
26		0.1376	0.1735	0.2269	0.2725	0.3242	0.3583	0.3889
27		0.1355	0.1710	0.2237	0.2686	0.3202	0.3543	0.3843
28		0.1335	0.1687	0.2208	0.2655	0.3163	0.3499	0.3801
29		0.1318	0.1664	0.2182	0.2622	0.3127	0.3460	0.3762
30		0.1300	0.1645	0.2155	0.2594	0.3093	0.3425	0.3718
31		0.1283	0.1624	0.2132	0.2567	0.3060	0.3390	0.3685
32		0.1268	0.1604	0.2110	0.2541	0.3036	0.3357	0.3646
33		0.1255	0.1590	0.2088	0.2513	0.2999	0.3323	0.3610
34		0.1240	0.1571	0.2066	0.2488	0.2973	0.3294	0.3583
35		0.1227	0.1555	0.2045	0.2467	0.2948	0.3266	0.3548
36		0.1215	0.1540	0.2026	0.2445	0.2921	0.3238	0.3522
37		0.1202	0.1525	0.2008	0.2423	0.2898	0.3213	0.3498
38		0.1192	0.1512	0.1993	0.2408	0.2879	0.3187	0.3465
39		0.1181	0.1499	0.1974	0.2383	0.2853	0.3163	0.3443
40		0.1169	0.1484	0.1958	0.2366	0.2836	0.3141	0.3415
41		0.1160	0.1472	0.1944	0.2350	0.2815	0.3124	0.3400
42		0.1153	0.1462	0.1930	0.2334	0.2794	0.3102	0.3377
43		0.1141	0.1449	0.1915	0.2319	0.2778	0.3081	0.3353
44		0.1134	0.1441	0.1902	0.2302	0.2758	0.3061	0.3332
45		0.1124	0.1430	0.1890	0.2288	0.2744	0.3050	0.3325
46		0.1116	0.1418	0.1875	0.2273	0.2726	0.3028	0.3298
47		0.1108	0.1408	0.1865	0.2257	0.2711	0.3009	0.3279
48		0.1102	0.1400	0.1850	0.2241	0.2690	0.2991	0.3256
49		0.1093	0.1390	0.1839	0.2228	0.2676	0.2972	0.3235
50		0.1087	0.1381	0.1829	0.2216	0.2662	0.2960	0.3225

Appendix C: Average, standard deviation, relative error, and coefficient of variation of ash percentage (unrounded values).

	Average	Standard Deviation	Relative Error	Coefficient of Variation
Ash %	2.602400233	0.2831866304	42.04008389	10.88174781