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ARTICLE

Carotenoids: The Colors Hold the Secret

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Given the growth of human population throughout the world, the amount of food required to feed everyone is of great importance, particularly in third world countries. This challenge is more acute when there is the need to have not only a sufficient amount of food, but also to have food which contains all the necessary nutrition for healthy eating. Canada has an important role to play with meeting this global requirement since nearly two-thirds of the world's supply of protein rich pulse crops is produced in Saskatchewan alone [Szalay, J.].

BACKGROUND

Lentils, an example of pulse crops, are a prime source of essential dietary nutrients such as proteins, vitamins, fibre and minerals (iron, potassium, selenium and zinc). In addition, carotenoids present in pulse crops act as antioxidants and have been shown to provide tremendous health benefits, such as fighting against degenerative diseases (ie: macular degeneration) and even some kinds of cancers [Szalay, J]. The carotenoids (beta-carotene, lutein, zeaxanthin, etc.) are a group of naturally occurring coloured nutritional compounds present within plants such as pulse crops, which contribute greatly to the nutritional value of legumes. Carotenoids have recently been gaining prominence, primarily by advancements in technology (i.e.: HPLC, mass spectrometry etc.) making it easier to measure and thus breed for them. Therefore, pulses are a valuable alternative to meat-based diets without compromising nutritional value. Additionally, these dry grains provide a huge advantage over perishable foods in handling and transportation, which allows them to reach across the globe to much needed and underserved geographic regions.

Most pulses require some type of cooking in preparation for consumption. Since boiling and frying are the two most common types of cooking methods worldwide, these cooking styles were used to treat the selected pulses in an effort to see what affect they may have had on the contained nutrients. This information is extremely valuable to both the consumer (people who eventually eat the food) and the scientists (the people to research and breed the food).

PURPOSE

This experiment will study the affect cooking has on the presence of valuable carotenoids in pulse crops. Six different samples representing chickpeas, lentils and peas will be cooked by both boiling and frying, including controls for each. The presence of carotenoids found in these foods will be evaluated (expression, value) to help understand the nutritional value of each crop for human health benefits.

HYPOTHESIS

Cooking is expected to have some effect on the concentration and/or quality of carotenoids found in selected pulse crops. It is possible that some legumes will be adversely affected, some positively affected and others not affected at all. This will have a direct impact on the remaining nutritional value, possibly changing the available benefits to human health. It is hypothesized this will occur because the heat from the cooking may modify (burn away, change chemical bonds, etc.) of the molecular makeup inside the crops.

PROCEDURE

Before beginning this experiment, the samples were prepared for testing by measuring out 50 grams of all legumes (peas, chickpeas, lentils) twice, with the aid of a digital weigh scale, and then hand grinding each specimen using a mortar-pestle grinder. Next, each sample was soaked in ¼ cup of (room temperature) water for 2 hours. When the soaking process was complete, the samples were strained out of water and dabbed onto paper towel to remove any remaining water. Next each sample was added separately to 1 cup of water in a medium sauce pan and place over medium heat (level 5.5) for 15 minutes. Once the cooking process was complete samples were removed from heat and strained out of water following placing onto filter paper to remove excess water. Samples were left to air dry for a 24-hour period followed by re-weighing each sample again once dry. Samples were hand ground, then packaged and labelled separately. Once ready to fry samples, each sample was added to 10 mL of coconut oil and then placed over low heat (3.5 level) for 10 minutes. When the cooking process was complete, samples were taken off heat and placed each onto filter paper to remove excess oil over a 24-hour period. To further remove excess oil, filter paper was repeatedly replaced (initially every 30 minutes, working up to a 2-hour interval or as needed). When drying/straining process was complete, each sample was re-weighed and measured, followed by hand grinding each sample as before. When hand grinding of all samples was complete, proceed to HPLC analysis. Prepared the HPLC machine by machine grinding all samples into fine powder. Next, prepared HPLC sample tubes by adding sample powder (0.01 micro gram) to 5 mL of solvent (methanol, acetone, dichloromethane, hydroxytoluene) in a micro test tube using a pipette. Placed the micro test tube into a micro centrifuge (11,000 RMP). Extracted the dissolved powder sample (1 mL) from the micro test tubes and loaded them separately into HPLC veils with the help of a mechanical pipette. Performed the HPLC testing procedure by loading the veils into a cryogenic storage rack tray and inserted it into the HPLC machine (Agilent 1200 LC, chemstation software). The automated program in the HPLC machine was then run. Finally, complete comparison analysis of the gathered data was compiled .

RESULTS

The results showed that both boiling and frying of the pulses, generally decreased the overall concentration of most carotenoids (Lutein, Zeaxanthin, Violaxanthin, Beta-Carotene). However, it is also of importance to note that boiling of the Channa Chickpea sample caused an increased expression of Violaxanthin, Zeaxanthin, Beta-Cryptoxanthin and Beta-Carotene.

The Green Split Pea sample showed interesting results. Boiling resulted in the Lutein concentration to be increased by 43% (compared to the raw state), frying caused Beta-Cryptoxanthin to be increased by 115% compared to the raw, while the Beta-Carotene concentration was decreased marginally by 8%.

Sample Name	Violaxanthin			Lutein			Zeaxanthin		
	RAW	Boiled	Fried	RAW	Boiled	Fried	RAW	Boiled	Fried
Red Lentil	33	0	0	1084	787	775	0	0	31
Green Lentil	37	0	0	1271	1154	626	144	85	69
Desi Chickpea	111	0	0	1641	1427	771	0	0	54
Channa Chickpea	0	128	0	1458	1237	1172	0	346	35
Green Split Pea	151	0	128	963	1375	1087	615	0	36
Yellow Split Pea	21	0	0	1101	111	864	0	0	63

Sample Name	Chlorophyll like Compounds			Beta-Cryptoxanthin			Beta-Carotene		
	RAW	Boiled	Fried	RAW	Boiled	Fried	RAW	Boiled	Fried
Red Lentil	0	0	0	0	0	0	0	0	0
Green Lentil	78	16	0	0	0	0	0	0	0
Desi Chickpea	0	0	0	0	0	0	0	0	0
Channa Chickpea	0	170	0	0	102	0	0	125	0
Green Split Pea	581	0	0	54	0	116	61	0	56
Yellow Split Pea	0	0	0	0	0	0	0	0	0

Table 1. Concentration of carotenoids in cooked and uncooked pulses.

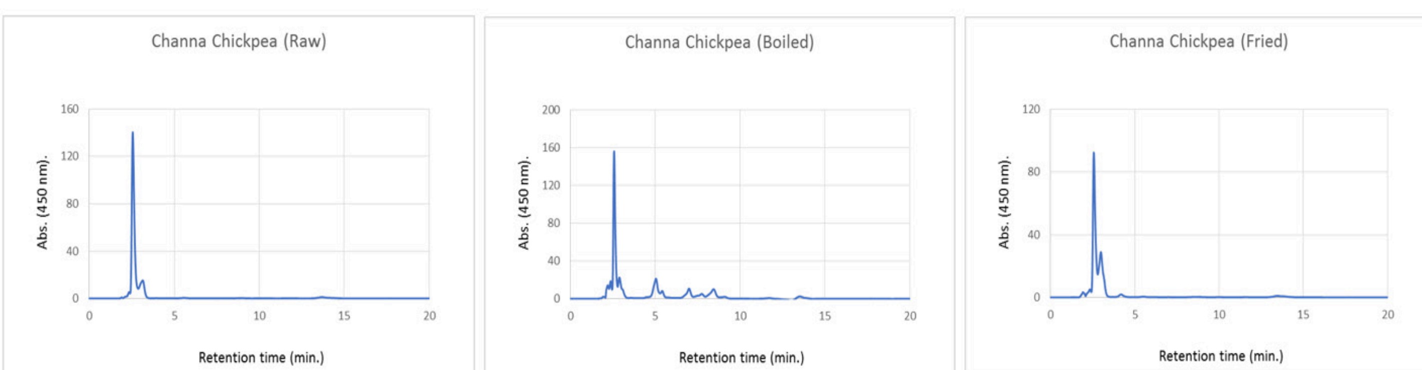

Figure 1. HPLC traces of (a) Raw (b) Boiled (c) Fried Channa Chickpeas

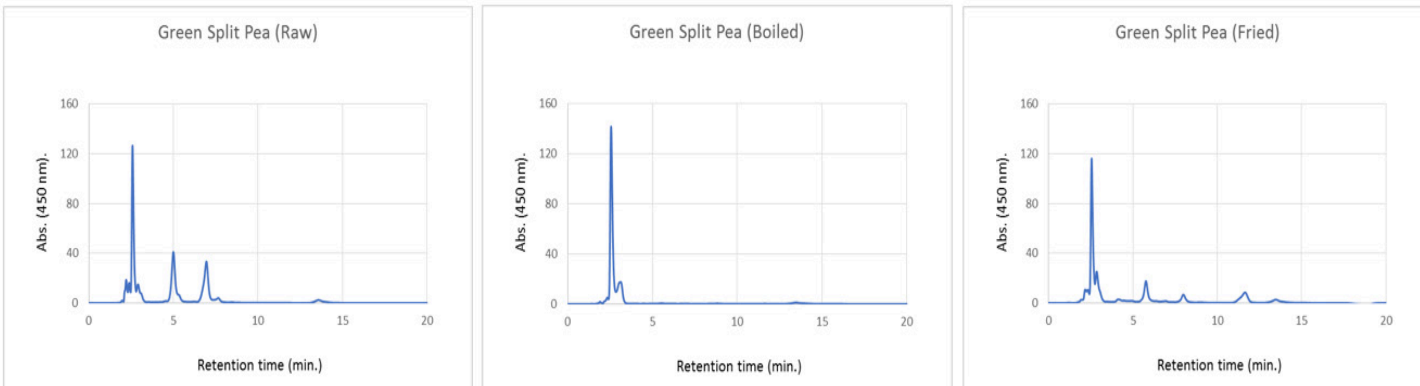

Figure 2: HPLC traces of (a) Raw (b) Boiled (c) Fried Green Split Peas.

	Lutein		
	RAW	Boiled	Fried
Red Lentil	1084	787	775
Green Lentil	1271	1154	626
Desi Chickpea	1641	1427	771
Channa Chickpea	1458	1237	1172
Green Split Pea	963	1375	1087
Yellow Split Pea	1101	111	864

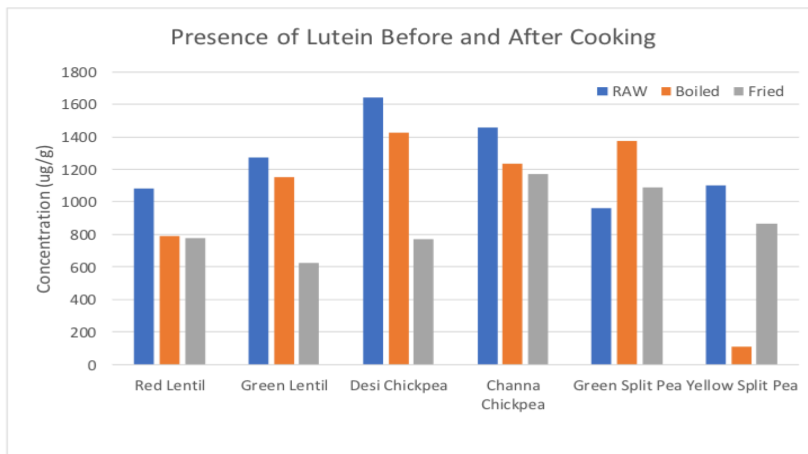

Figure 3. Presence of Lutein Before and After Cooking.

	Zeaxanthin		
	RAW	Boiled	Fried
Red Lentil	0	0	31
Green Lentil	144	85	69
Desi Chickpea	0	0	54
Channa Chickpea	0	346	35
Green Split Pea	615	0	36
Yellow Split Pea	0	0	63

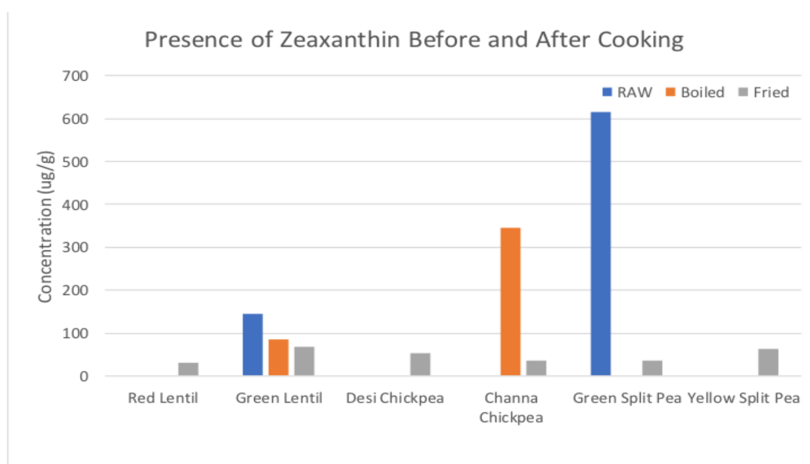

Figure 4. Presence of Zeaxanthin Before and After Cooking.

DISCUSSION

The purpose of this experiment was to study the affect cooking has on the inherent carotenoid concentrations found in Saskatchewan-grown pulse crops, specifically, lentils, chickpeas and peas. It was hypothesized that both boiling and frying of the selected crops would have some affect, which may have been negative, positive or non-existent. The motivation behind the project was to gain a better understanding of human health benefits vis-à-vis how the nutritional value could be altered by the cooking process.

The outcome of the experiment showed that the hypothesis was true. While it was not entirely clear at the beginning of the project which carotenoids would be found, and of those present, which ones would be affected and to what degree, it was anticipated some change would be observed. After reviewing the results, it is proposed frying (in oil) had a more harsh effect on the pulses because of the direct and more immediate heat. It stands to reason that frying heat would cause the contained moisture to be lost from the legumes. This was clearly evidenced by the decrease in weights, especially among the fried samples. Channa Chickpea showed a reduction of 24% in volume weight.

The relevance of these results is that, firstly, frying has a greater adverse effect on the edibles compared to boiling. Accordingly, the associated nutritional health benefits are also equally reduced when they are fried. So, while pulse crops offer great practicality for handling and contain valuable health benefits, to capture the maximum positive impacts, the identified foods should be boiled and not fried in preparation for consumption. Additionally, this also points a guiding light for researchers and scientists to place a greater focus on further developing the identified chickpeas, lentils and peas to have an increased ability to withstand heat damage from frying in order to retain their valuable nutrition content.

Similar to any other experimental trial, this project was no exception to being influenced by different types of variables, namely, independent, dependant, controlled and constant. Independent variables included such items as the weight of the samples, the cooking heat, the cooking time, the water soak time and grinding of the samples. By contrast, dependent variables were the concentrations of carotenoids and the change in weight of the samples due to cooking. The controlled variable in the experiment was the one sample which were prepared the same way as all the other samples but was not exposed to cooking, neither boiling nor frying. This included one sample of the lentils (green and red), one sample of the split peas (green and yellow) and once sample each of the chickpeas (Channa, and Desi). Finally, the observed constant variables were time, humidity, toom temperature, exposure to light and the HPLC measurement protocol.

There are two major potential sources of error which need to be acknowledged with the experiment; human errors and systematic errors. The possible human errors which could have had some effect on the experiment, especially with the handling and cooking of the samples, were; getting finger oil on the samples when they were being handled, accurate measurement of liquids such as water and oil, cleaning of the mortar and pestle hand mill between grinding different samples, accidental spillage of sample grinds, inaccurate measurement of each sample when weighing, possible cross-mixing of ground up samples (powder form), lack of thorough cleaning of cooking utensils (pots, pans, spatulas, etc.), keeping the cooking time exactly consistent between different samples, lack of consistent adherence to procedure between trials, interpretation of observed results (HPLC graphs, visual inspection of oil and/or water), failure to thoroughly remove remaining oil and/or water from samples after cooking, and accurate record keeping when recording data. Equally, systematic errors could have also had an effect on the results of the experiment. Some of these included; calibration of the HPLC machine, calibration of the digital weigh scale, impurities present in the tap water, impurities in the cooking oil, uneven or delayed heat generated by the stove and change in the room atmosphere (temperature, humidity, light, airflow, etc.).

FUTURE IMPROVEMENTS

As in with any scientific trial, unfortunately the chance of errors effecting the results of the experiment will never be reduced to zero. For future trials some changes can be considered to minimize the errors as well as the impact caused by errors to hopefully give better and more reliable results. Some changes for future improvements include; using a laboratory grade grinder to grind up the samples instead of using a mortar pistol hand mill; using an electronic timer to turn the stove on and off; using a professional dehumidifier to dry all the samples; using a professional digital weigh scale for very accurate measurements, storing the raw and prepared samples in a dark room where there is no natural and artificial light, conducting the experiment in a climate-controlled room for temperature, humidity, air flow, and using super absorbent drying cloths to remove maximum amounts of water and oil.

CONCLUSION

The findings of the experiment showed the hypothesis was true, as both boiling and frying of the pulses generally caused a decrease in the overall concentration of most carotenoids (Lutein, Zeaxanthin, Violaxanthin, Beta-Carotene). Additionally, it is also of interest to note that boiling, specifically of Channa Chickpea, netted an increased expression of Violaxanthin, Zeaxanthin, Beta-Cryptoxanthin and Beta-Carotene. Also, the Green Split Pea sample demonstrated very interesting results. Boiling resulted in the Lutein concentration to be increased by 43% (compared to the raw state), frying caused Beta-Cryptoxanthin to be increased by 115% compared to the raw, while the Beta-Carotene concentration was decreased marginally by 8%.

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AFFAAN ABID

My name is Affaan Abid and I am a 13 year old student in Saskatoon, SK. I enjoy learning about many topics related to science especially with hands-on activities so I can experience what I am learning, by doing. Some of my hobbies include math, competitive basketball, hockey, puzzles and playing the alto saxophone. Additionally, I enjoy participating in science fairs and math competitions. I learned a great deal of knowledge from my project this year, specifically on how it related to human health benefits. I received the original idea to study carotenoids after researching on vitamin A. In the future I hope to continue investigating science-based solutions to bring about a better quality of life for all people around the globe.