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Increasing the resistant starch content of French Fries using dehydration and retrogradation.

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Abstract

French fries are a high-glycemic food that is ubiquitously available and excessively consumed. This study utilized the processes of dehydration and retrogradation of potatoes before frying to increase the content of resistant starch (RS) in French fries. RS is not digested or absorbed in the upper gastro-intestinal tract and passes to the large intestine where it is fermented into largely beneficial products by the microbiome. An increase in RS potentially translates into a lower-glycemic index and calorific value, thereby mitigating the onset and symptoms of metabolic disease.

Introduction

Resistant starch refers to starch that is not digested and absorbed in the upper digestive tract and passes into the large intestine where it is fermented by the residual microbiota to yield (among other products), short chain fatty acids (SCFA). Foods high in RS have a lower glycemic index, the RS fraction has been shown to increase insulin sensitivity, and decrease satiety related enzyme biomarkers such as Leptin (1). Studies are ongoing to ascertain whether sufficient consumption of RS can alleviate metabolic diseases such as diabetes and obesity (2). SCFA production has (generally favorable) implications in immunomodulatory, inflammatory and metabolic pathways (3). Taken together, the literature suggests that the consumption of RS may have health benefits.

French fries have become ubiquitous components of fast-food 'meals'. The amount of RS in french fries is significantly lesser than that found in retrograded or non-fried potato foods such as potato salads or baked potatoes. The frequent consumption of fried potatoes has been reported to be associated with an increased mortality risk (4). Therefore, the problem of how to increase the RS content of French fries, while substantially retaining the same manufacturing process and taste, seems worthy of pursuit.

Materials and methods

Sodium sulfite, lot # 233269, potassium sodium tartrate (Rochelle salt), lot # 230594, corn starch, lab grade, lot # 119062, starch solution, lot # 124721, Fungal Amylase (*A. oryzae*) lot # 211315, Benedict's qualitative solution, lot # 117957 were obtained from Flinn Scientific, Batavia, IL. 3,5-Dinitrosalicylic acid, lot # MKCC2916, was obtained from Sigma-Aldrich, St. Louis, MO. Iodine, Starch Test, lot # AD-11192-2, was obtained from Aldon Corporation, Avon, NY. Dextrose, lot # AD-13360-13, Sodium Phosphate

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dibasic, lot # AD-13164-1, Sodium Phosphate monobasic, lot # AD-13298-44 were from Carolina Biological Supply, Burlington, NC. Sodium hydroxide, lot # AD-13261-10, was from Ward's science, Rochester, NY

Russet potatoes and Great Value™ Vegetable oil (multiple lots) were obtained from the local Wal-Mart grocery store. The potatoes were used within one week of purchase. The vegetable oil, packaged in a 1 gallon plastic bottle, was used within one month of opening the bottle. Fresh Vegetable oil was used if the time elapsed since preparing the last batch was > 24 hours. An electric fryer – Fry Daddy™ elite, National Presto Industries INC., Eau Claire, WI, was used to fry the cut potatoes. Materials more than 1 g were weighed on a Flinn Scientific balance, model 0B2141, d=0.01 g. When < 1 g material was required, they were weighed on an Ohaus Pioneer™ balance, model PA153, d= 0.001 g, Ohaus Corp., Parsippany, NJ. City water direct from faucet was used for all aqueous preparations.

Preparation of the dinitrosalicylic acid (DNS) reagent

1 gram of dinitrosalicylic acid was added to a beaker containing 90 mL of water on a stirring plate. Then 0.05 grams of sodium sulfite was added. After dissolution, 1 gram of sodium hydroxide was added and dissolved. The beaker and contents were covered with aluminum foil and left stirring overnight. The solution was then filtered through a qualitative filter paper and poured into a 100 mL volumetric flask. Water was added quantity sufficient (Q.S). The DNS reagent was stored at room temperature and prepared as needed.

Preparation of Rochelle salt solution

20.0 g of sodium potassium tartrate was dissolved in sufficient water and the volume made up to 50 g in a suitably sized conical flask.

Preparation of standard solutions of dextrose

A 2% w/w solution of dextrose in water was prepared by dissolving 2 g dextrose to 100 g with water in a volumetric flask. Separate aliquots of 2.5 g, 1.276 g, 0.634 g, 0.312 g and 0.175 g of the 2% w/w dextrose solution were weighed into separate test tubes. Into each test tube was added 5 mL of the DNS reagent. The test tubes were covered with aluminum foil and immersed in boiling water for 5 minutes. The solutions were removed from the boiling water, and 1 mL of the Rochelle salt solution was added to each. Each solution was then diluted to 25 g with 0.1 M phosphate buffer at pH 7.0. The absorbance of these solutions were measured using a Flinn Scientific spectrophotometer, model FLU1503075, Flinn Scientific, Batavia, IL - at a wavelength of 575 nm. A previous experiment using 3 mL of DNS solution and 1 mL of Rochelle salt solution diluted to 25 g with phosphate buffer as a reference, showed no absorbance at this wavelength.

Cutting procedure

It has been previously reported that the surface to volume ratio of cut potatoes is a factor in determining the resistant starch remaining after frying (5). Potatoes were peeled and cut along the axial (length) plane only. Each potato was cut in half. Each half section was then cut into 4 sections. The sections were then laid flat and each was cut axially into 8 fries. The width and height of each fry measured approximately 1 cm X 0.6 cm.

Frying procedure

The Fry Daddy™ was filled to the 'minimum' level marked on the container with vegetable oil. At this level, the volume of the oil was 740 mL. A small piece of cut potato was immersed in the oil until the potato floated to the surface of the oil. At this time, the oil was hot enough to start the experiment. Each batch consisted of

200.0 ± 10.0 g of cut potatoes. The batch of potatoes were put in all at once in the oil and stirred periodically until they appeared golden brown (7 ± 1 minutes of frying). They were then removed from the oil and spread on a sheet of napkins to drain the excess oil.

Retrogradation procedure

Gelation of potato starch was performed in a 'pressure cooker', Prestige™ deluxe, 5.5 L capacity, TTK Prestige Ltd., Bengaluru, India, by heating the peeled and axially half cut potatoes with steam until a 'whistle' count of 58. The steam outlet was closed with the provided pressure weight when a continuous stream of steam emanated from the steam outlet. The number of 'whistles' (due to the weight being raised by the steam pressure inside the cooker) were then counted, rather than the time of cooking to account for differences in heating rates between different heating appliances (gas stove-top and electric heating plate). The time between 'whistles' was ~ 10 s, thereby yielding a total time for gelatinization of ~ 10 minutes (counted from the first whistle). The potatoes were stored elevated in the pressure cooker so that they did not contact the residual water in the cooker. Previous experiments demonstrated gelation of starch granules throughout the potato interior at this time (figure 1). The potatoes were then individually wrapped in aluminum foil and placed in a refrigerator (4°C – 8°C) for 10 ± 2 hours. Retrogradation of the gelatinized starch was assumed to be complete under these conditions (6). The potatoes were then cut and fried after equilibration to room temperature (< 1 hour).

Figure 1: Time, measured as the number of 'whistles' required for complete gelation of potato starch

Dehydration procedure

Dehydration of potato starch was performed in a 'pressure cooker', Prestige™ pressure cooker. The potatoes were peeled and cut as previously described. 440 g of the cut potatoes were placed in a 'scaffold pattern' inside the pressure cooker and subjected to a vacuum for 75 minutes by connecting a single stage rotary vacuum pump (Model VP3.0, Mountain vacuum products, Montrose, CO, USA) to the pressure cooker's steam outlet and reversing the direction of the pressure release safety valve. The pressure inside the cooker was not measured but could be speculated to be < 1 mm Hg based on the vacuum pump specifications guide. The potatoes lost an average of 6.5 ± 1.0% of their initial weight after the process. The potatoes were immediately subjected to the frying process after this procedure.

Figure 2: Arrangement of cut potatoes in pressure cooker before pressure reduction.

Measurement of resistant starch

There are several methods available to measure RS content. The results obtained are not comparable and depend on the method used (7). The method used in this work utilized a fungal amylase enzyme to digest the starch for a predetermined time (see below). This was followed by the separation of the NRS starch from a mixture of RS and NRS starch. The two fractions were then completely converted to glucose by acid hydrolysis at elevated temperature. Preliminary experiments demonstrated that this procedure allowed for a measureable relative difference between the RS and the total starch (NRS+RS) fractions using UV-VIS spectrophotometry.

3 mL of 2.5% starch solution (corresponding to 0.025 g starch per mL of gastric fluid, see below) with or without 0.04 g of amylase enzyme was incubated in horizontal corked test tubes with constant shaking in an oven maintained at 37^{o}C. A test tube was taken out of the oven at 5, 15, 60 and 120 minutes and contacted with 1 mL of Benedict's reagent. The test tube was heated in a boiling water bath for 5 minutes and the color of the precipitate was examined (figure 3).

Figure 3: Determination of starch digestion time by amylase

This time (2 hours) was taken to be the time at which the enzyme converted enough starch into reducing sugars (not necessarily into monosaccharides) so as to provide a measurable relative difference between the resistant and non-resistant starch for French fries made using the different methods. This incubation time matched the time of passage of food constituents through the stomach and was considered enough to digest the rapidly digesting starch as well as the slowly digesting starch fractions (8). The ratio of starch to water was of the same order of magnitude, but lesser than, the consumption of one order of large French fries (150 g containing 20% starch) in 700 mL of gastric fluid (0.04 g starch per mL of gastric fluid) under fed conditions. Consequently, subsequent experiments were performed for this time period.

French fries were prepared using raw (as is), dehydrated or retrograded potatoes. The prepared fries were contacted twice (using fresh paper towels each time) to remove excess oil. 20 ± 2 g of fries were then minced using a Ninja™ Express Chop, NJ100 Series, SharkNinja Operating LLC, Newton, MA for < 10 seconds. This time period was sufficient to uniformly grind the fries yet not too long so as to cause the ground fries to agglomerate into a dough. A 2.0 g of minced sample was removed and dispersed gently (< 2 minutes) into a homogeneous dispersion using 10 mL of water in a ceramic mortar and pestle. 7 mL of the dispersion was then transferred to an appropriately sized test

tube. Into this starch dispersion was added 0.1 g of amylase enzyme. The contents of the test tube, containing (a theoretical amount of) ~0.04 g starch per mL, were shaken in an oven for 2 hours at 37° C to mimic the action of amylase on the starch in the gastro-intestinal system. The 2 hour time was chosen from the starch digestion experiment (see above) with Benedict's reagent. The denaturation temperature of the fungal Amylase enzyme used in the study was visually determined in a 0.1% solution in water as > 60°C.

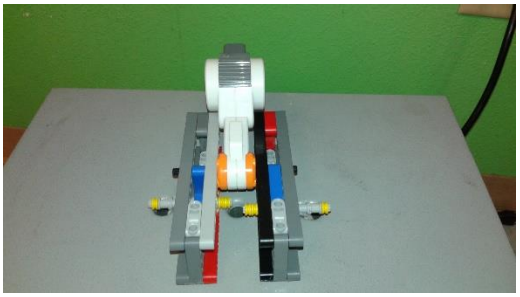
While stirring, 2 g of the starch dispersion was withdrawn into a test tube and 2 g of 6 M, HCl was added. This tube was labeled (NRS+RS), signifying that it contained the sum of the resistant and non-resistant starch. Another 3 g sample was taken from the same starch dispersion and subjected to centrifugation for 1 hour at a Relative centrifugal force (RCF) of 3354 g, using a RevSpin RS-200T microcentrifuge, Revolutionary Science, Shafer, MN. After centrifugation, a 2 g aliquot of the supernatant (combined from the centrifuge tubes) was withdrawn into a separate test tube and 2 g of 6 M. HCl was added. The tube was labeled (NRS) to indicate that it contained the non-resistant starch only. Both test tubes were incubated for 2 hours in a 95° C oven with constant shaking using a shaking device made from lego™ Mindstorms™ and a sine wave generator (see below for description). The literature indicated complete hydrolysis of starch at this time, temperature and acid concentration (9). After the incubation period, the test tubes were removed from the oven and the contents of each test tube was neutralized with 2 mL of 6 M, NaOH.

Device for sustained agitation of test tubes within a heated environment

A test tube rack was suspended in the oven from 4 twine strands of equal length, connected to opposite sides of an oscillating apparatus through vent holes. The oscillating apparatus used was constructed of Lego™, controlled by a Lego™ MindStorms™ motor, driven by a Pasco (PASCO Inc., Roseville, CA) 15 Volt Sine wave generator [Model #WA9867] tuned to 2.0 Hz at 4.0 V (tuned to 4.0 V by tuning the Pasco Sine Wave Generator to 60.0 Hz and measured with an AC voltmeter). Photographs of the device are presented in figures 4 and 5.

Figure 4: Test tube shaker, front view

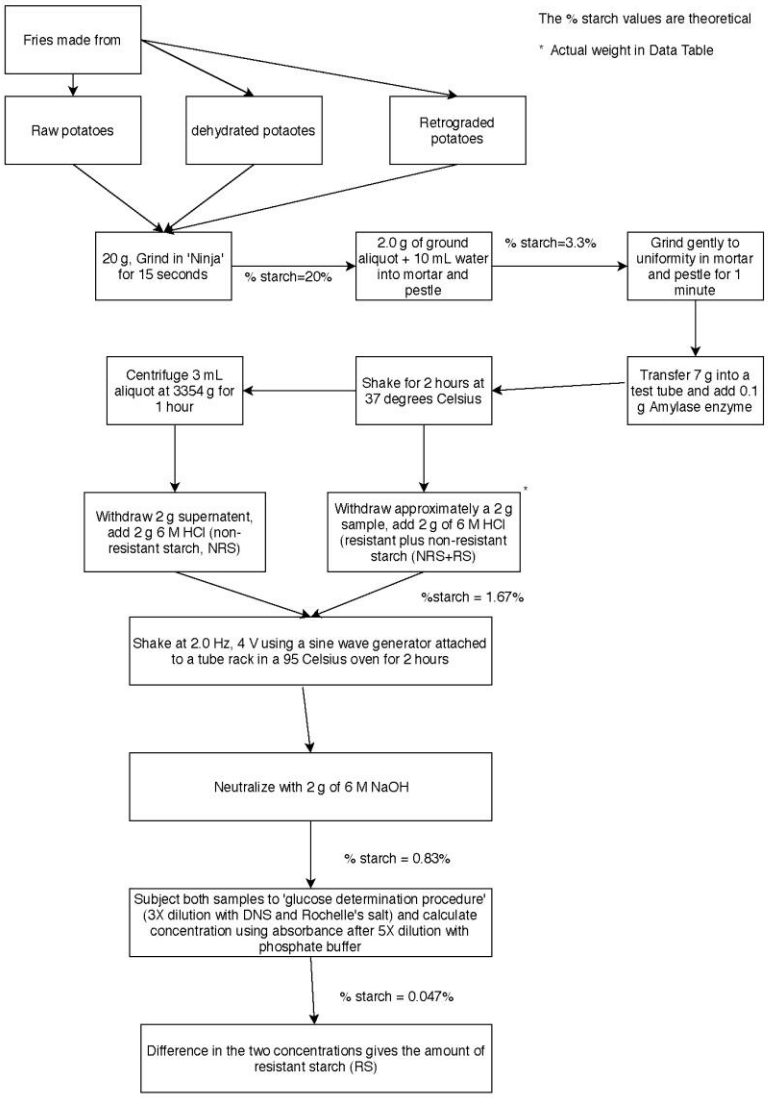
Figure 5: Test tube shaker, top view



Glucose determination procedure

After incubation, hydrolysis of all the starch and/or the starch oligomers to the reducing monosaccharide glucose and neutralization, a 2 g aliquot was withdrawn from the test tube and 4 g of DNS reagent added. The test tube was heated in a boiling water bath for 5 minutes, after which time 1 mL of Rochelle salt solution was added. The amount of dextrose was determined after a 5X dilution with 0.1 M, Phosphate buffer solution at pH 7.0. The final dilution was performed with the buffer because the pH of the NaOH neutralized solution could not be measured without a significant loss of sample. Consequently, phosphate buffer was added to ensure that the pH of all the samples immediately prior to the absorbance measurement step was the same.

Figure 6: Flowchart of the study design



Results and discussion

Glucose reacts with DNS under alkaline conditions in a 1:1 molar ratio, reducing the latter to 3-amino-5-nitro salicylic acid; which absorbs light at 575 nm (10). The glucose oxidizes to form gluconic acid as shown in the reaction below.

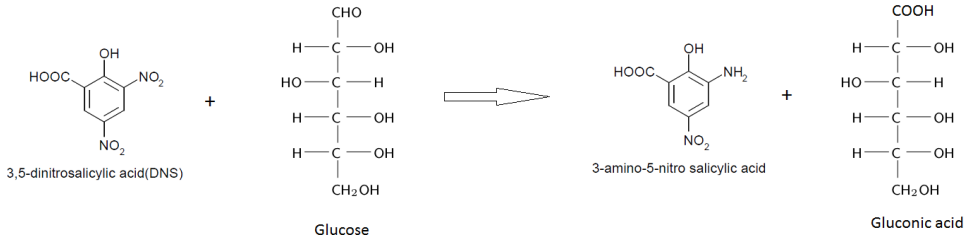


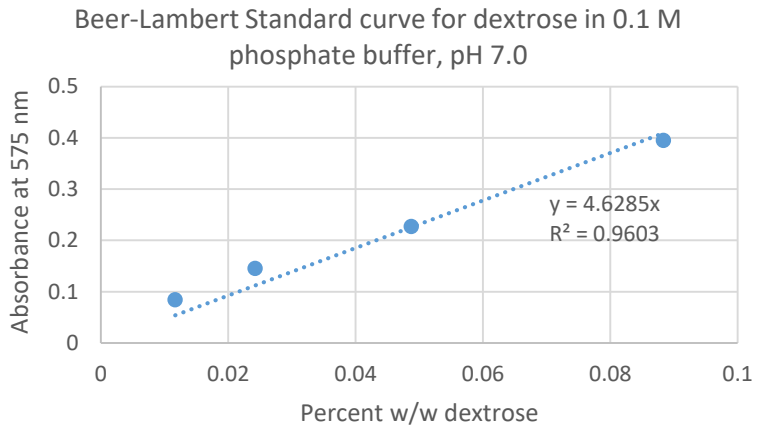
Table 1 shows the stoichiometric ratios used to generate the standard curve for the determination of glucose in water. The standard curve using the 5 dextrose concentrations in the table was not linear (R^2>0.82). This was because, at the highest dextrose concentration, the DNS became the limiting reactant thereby preventing more of the reduced colored product (3-amino-5-nitro salicylic acid) from being formed and leaving the molar excess of dextrose unreacted. When this greatest dextrose concentration was excluded from the standard curve, the curve was linear (R^2> 0.99), in conformity with either a stioichiometric equivalent or excess of the DNS reagent with respect to dextrose concentration. The standard curve in figure 1 was hence generated using the data points where the DNS was in molar excess or in stoichiometric equivalent to dextrose. This curve was generated in 0.1 M phosphate buffer, pH 7.0. Another standard curve generated by another student on a separate day obtained an R^2 > 0.97 (data not shown) and the equation y= 4.3142 X. The inter-day variability, expressed as the relative standard deviation (RSD) of the mean of the slope in standard curves was hence 4.9%. All subsequent experiments utilized a stoichiometric excess of DNS.

Table 1: Standard curve for dextrose, stoichiometric data

Dextrose solution[1] used (g)	DNS solution[2] used (mL)	Dextrose (moles X 10^5)	DNS (moles X 10^5)	Absorbance[3]
0.175	3	1.94	14.14	0.134
0.312	3	3.46	14.14	0.247
0.634	3	7.04	14.14	0.480
1.276	3	14.16	14.14	0.851
2.5	3	27.75	14.14	0.998

1. Taken as aliquot from the 2% w/w stock Dextrose solution. The greatest dextrose concentration corresponding to the last row was subsequently excluded from the standard curve as explained in the text.
2. Taken as aliquot from the 1% w/v stock DNS solution, using a 10 mL measuring cylinder. Subsequent experiments utilized a 5 mL volume to ensure stoichiometric excess.
3. After heating in boiling water for 5 minutes, adding 1 mL Rochelle salt solution and diluting to 50 g. Measured at 575 nm. (see procedure for details)

Figure 7: Standard curve for dextrose



Raw potatoes contain from 16 to 24% starch by weight as-is (11) or about 80% by dry weight (5). Theoretically, the NRS + RS starch content would have been diluted down to 0.047% (starch) at the end of the starch determination procedure as shown in the flowchart. This concentration is at the order of magnitude of dextrose used to generate the standard curve.

Dehydration and retrogradation have been shown to increase the percentage of resistant starch in potatoes (12 and references therein, 13). Therefore, these two processing conditions were chosen to attempt to increase the RS content in French Fries. Inaccessibility to thermal analysis and viscosity measuring instrumentation did not allow us to measure and use predetermined heating/cooling rates, temperature ranges (14), moisture contents (15) and annealing cycles (6) so as to allow for, and provide evidence of, maximum retrogradation (14). Other reported methods to increase the RS content such as baking or roasting instead of frying, modulating the amylose to amylopectin ratio, using additives, or chemical modifications were not investigated in this study because the objective was to increase RS without extensive changes to current manufacturing processes and without extensive taste alteration.

Table 2: RS and NRS starch in French fries made by using raw, dehydrated and retrograded potatoes

French Fries made from	NRS starch (grams*, absorbance**, percent NRS starch***)	NRS+RS starch (grams*, absorbance**, percent NRS + RS starch***)	%RS starch, as A percentage of Total starch
Raw potatoes	2.133 g, 0.312 (17.44%) 1.943 g, 0.312 (18.34%)	2.223 g, 0.525 (28.58%) 2.2 g, 0.524 (28.58%)	11.14%, 38.9% 10.24%, 35.82%
Dehydrated potatoes	2.04 g, 0.191 (11.14) 2.06 g, 0.225 (13.13%)	2.01 g, 0.402 (23.45%) 2.00 g, 0.410 (23.45%)	12.31%, 52.49% 10.32%, 44.02%
Retrograded potatoes	2.38 g, 0.247 (12.87%) 2.127 g, 0.296 (16.58%)	2.0 g, 0.663 (38.68%) 2.0 g, 0.641 (37.39%)	25.81%, 66.73% 20.81%, 55.66%

NRS + RS starch = the sum of non-resistant and resistant starch, NRS=non-resistant starch
*The actual amount weighed out at the starred process box in the flow-chart, figure 6.
**The absorbance of the diluted solution at the end point of the process in figure 6, flow-chart.
***Starting concentration of the particular type of starch.

Table 2 shows the results of the RS in French fries made using the three different methods. The two rows for each procedure represent the results of two different experiments.
Data analysis was performed using a one-way ANOVA with a Tukey HSD post-hoc test at statpages.info/anova1sm.html. Results were considered significant at $p<0.05$. The retrogradation procedure significantly increased the percent of RS when compared to the raw potato group with a p value = 0.05. The percent RS between the dehydrated and the raw potatoes group was not significantly different ($p=0.29$). There was also no significant change in the percent RS between the dehydrated and the retrograded group ($p=0.21$).

The values for the NRS+RS were reproducible, whereas those for the NRS were less so. The % RSD for NRS was 3.5%, 11.6% and 17.8% and that for the NRS+RS was 0%, 0% and 2.4% for the fries prepared from the raw, dehydrated and retrograded potatoes respectively. The authors speculate that the larger variation in the NRS values could be due to the a less than optimal shaking procedure for the corked test tube placed in the horizontal position containing the homogenized French fry dispersion with the amylase enzyme. This occurred despite the fact that the enzyme was dispersed throughout the test-tube contents with a glass rod before incubation. Another source of error could be the inadvertent drawing up of part of the precipitate into the pipet after centrifugation, although reasonable care was exercised during this procedure.

There are admittedly several steps in the procedure where there is the possibility of changing the ratio of RS to NRS starch. For example, the grinding procedure of the fries in the Ninja as well as the dispersion of the ground fries in the mortar and pestle, could theoretically provide shear forces to overcome the intermolecular forces (IMF) between polymer chains (16). However, the time of these steps was kept as short as possible and fries made using raw, dehydrated and retrograded potatoes were subjected to the same steps. This ensured that the relative difference between the percent RS found in fries made from raw, dehydrated and retrograded potatoes would remain unaffected, assuming equal propensity of IMF perturbation.

Unlike gravimetric methods (17), an advantage in using the DNS method for the determination of glucose was that it was insensitive to, and invariant of, the fat and protein content of the potatoes. Therefore, de-fatting and de-proteination procedures were considered unnecessary for this method of determination of NRS and RS starch. The values for RS reported in the literature vary widely due to the differences in methodology. For example, only 1% RS was found in French fries (5) using a method that incubated the sample with amylase for 16 hours; 8 times greater than the incubation time in this work. The variability in the results reported in the literature using different protocols underscores the importance of standardizing the methodology as well as making it representative of *in vivo* gastrointestinal motility parameters.

Conclusions

The percent RS in French fries could be significantly ($p \leq 0.05$) increased using potatoes that had been previously gelatinized and retrograded. The dehydration procedure as used in this study could not significantly increase the French fry percent RS content.

Further studies will investigate the effect of these process conditions on the taste of the prepared French Fries. The percent RS produced by these methods will also be compared to that produced by the manufacturing process used by McDonalds Inc., especially because McDonalds uses a Par-fry and freeze (to -12^{o}C) method before shipping to restaurants, that has the potential to increase the resistant starch content (18). It will also be of interest to determine if a combination of these processes; i.e. dehydration followed by retrogradation, results in an additive or synergistic

RS increasing effect while retaining the distinctive taste and texture of French fries.

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