The Journal of Plastination 32(1): 26-30 (2020)

ORIGINAL RESEARCH

Dezse, K. E.
Frank, W. P.
Baptista, C. A. C.*

Department of Medical Education, College of Medicine and Life Sciences University of Toledo, 3035 Arlington Avenue, Toledo, Ohio 43614.

Fat Removal during Acetone Dehydration and Defatting Phases of Plastination

ABSTRACT:
It is well known among plastinators that acetone is a good degreaser when used at room temperature. In fact, the dehydration process is achieved by using acetone at -25º C, followed by a degreasing process utilizing acetone or dichloromethane at room temperature. The objective of this study was to identify the rate and effectiveness that cold acetone (-25º C) has on fat removal during the dehydration process and to compare it with the defatting phase at room temperature. Samples were soaked in -25º C acetone for 21 days (3 baths of 100% acetone) and then 20º C for 21 days (3 baths of 100% acetone). Acetone was changed at 7-day intervals and the "Dirty"/used acetone was collected and total fat content was determined using a rotary evaporator. Over a 42-day period, the samples demonstrated a uniform pattern of decreasing fat extraction during dehydration. However, when degreasing was initiated, a sharp increase of extraction was observed which ended with a steep decline. These findings were supported by mirrored purity readings with an acetonometer. Rate of extraction was greatest during the first seven days of both phases. Dehydration yielded 17.9 % more fat extracted by weight when compared to the defatting phase. This study represents the first report that the dehydration phase may play a more important role in degreasing than the defatting phase itself. Using acetone purity readings with calculated k-values for average rate of extraction data points allows one to quantify and determine a "successful extraction of fat."

KEY WORDS: fat; dehydration; defatting; degreasing; rotary evaporator; plastination

***Correspondence to: Carlos A C Baptista, Department of Medical Education, College of Medicine & Life Sciences, University of Toledo, Health Science Campus, 3035 Arlington Avenue, Toledo, Ohio 43614. Email: carlos.baptista@utoledo.edu**

Introduction

White adipose tissue (WAT) and brown adipose tissue (BAT) are the two most common tissues that house adipocytes in mammalian species (Avram et al., 2005). They both function to alter energy metabolism but opposite of one another. WAT sequesters energy left over from food consumption and transforms it into stored lipids while BAT breaks down these stored lipids in a process known as thermogenesis to produce heat (Verma et al., 2016). Their lipid configuration also differs, WAT has a large singular droplet whereas BAT displays smaller multinodular droplets (Wu et al., 2012). A hybrid exists between these two types, beige adipocytes, and as the name suggests, its function is a mix between WAT and BAT. Beige cells express uncoupling protein 1, (UCP1) (gene that allows adipocytes to break down lipids into thermal energy), the same as brown cells but not with the same efficacy as brown cells. Plastination is the process of removing water and fat from tissue in order to impregnate the tissues with silicone, epoxy, or polyester. In order to remove the water and fat content from cells, a dehydrating and a defatting agent is used (von Hagens, 1985). Methanol, isopropyl alcohol, dimethyl sulfoxide, and acetic acid, all show average penetration through the lipid cell membrane (Young et al., 2010). Acetone and dichloromethane show the most penetration, and consequently the most lipid extraction (von Hagens, 1985; Henry, 2007). At room temperature, acetone is the

most widespread defatting agent used in plastination labs. It is known that a small amount of degreasing occurs during the dehydration step (von Hagens, 1985; Henry, 2007; DeJong and Henry, 2007).

Acetone is an excellent dehydrating agent when used at -25ºC. Acetone acts as a formidable defatting agent when used at room temperature. It is well known in the plastination community that differently from the dehydration phase, the defatting phase of plastination is not determined by quantitative measurements. The degree of dehydration of a specimen is determined using an acetonometer that measures indirectly the quantity of water that is extracted from the sample (von Hagens, 1985). In the defatting phase samples are soaked in acetone for varying intervals of time. Discoloration of the acetone bath is the determining factor that dictates when and how often the defatting acetone bath should be changed. This process is imperfect and time consuming and may lead to a poorly defatted sample. To minimize this inevitable error, measurements were taken to determine the rate of fat extraction in a given sample, which was corroborated by acetone purity readings via an acetonometer. Additionally, the overall effectiveness of the acetone as a degreaser was measured by collecting the fat released after a given time interval. In this report, we identify the rate and effectiveness of extraction that acetone has on fat tissue during the dehydration and defatting phases of plastination.

Materials and Methods

Four uniform skin flaps measuring 3 in3 (49 cm3) were collected from the same cadaver and a similar location on the body. It is known that the lower back is an ideal location to obtain a fatty specimen. The samples were excised from the body and fixed in 10% formalin (DeJong and Henry, 2007) for a period of seven days. After fixation, the fat was dissected away from the skin to ensure only adipose tissue was being dehydrated. Each sample was weighed and placed in a 1L aliquot of cold (-25ºC) 100% acetone. Each acetone aliquot was changed with a fresh 1L of 100% acetone at 7, 14 and 21 days. The dirty acetone was measured with an acetonometer to determine acetone purity.

Each of these “dirty acetone” mixtures were processed in a rotary evaporator to determine the total amount of fat released by the sample for that given week (soak period). When it was concluded that no additional water could be extracted (acetone density readings were >99% pure), the samples were placed in fresh acetone vats at 20°C (the beginning of the defatting phase).

In the defatting phase, the samples were assigned another 21-day soak period at room temperature. At 7-day intervals, the samples had their dirty acetone collected and vats filled with a fresh liter of new acetone. Density and total fat extracted (via rotary evaporator) measurements in grams (g) were recorded from each weekly sample.

DAY	Sample #1	Temp.	Density	Fat Extracted (g)	% of Fat Extracted
7	Dehydration 1	-25 C	98.0%	23.9704	22.37%
14	Dehydration 2	-25 C	98.6%	16.6504	15.54%
21	Dehydration 3	-25 C	99.2%	12.627	11.79%
	Total			53.2478	49.70%
28	Defat 1	20 C	98.1%	38.7566	36.17%
35	Defat 2	20 C	98.9%	8.9958	8.40%
42	Defat 3	20 C	99.3%	0.7986	0.75%
	Total			48.551	45.32%
DAY	Sample #2	Temp.	Density	Fat Extracted (g)	% of Fat Extracted
7	Dehydration 1	-25 C	98.8%	22.1852	29.90%
14	Dehydration 2	-25 C	99.0%	14.775	19.92%
21	Dehydration 3	-25 C	99.4%	6.6054	8.90%
	Total			43.5656	58.72%
28	Defat 1	20 C	98.5%	25.1512	33.90%
35	Defat 2	20 C	99.1%	2.8668	3.86%
42	Defat 3	20 C	99.3%	0.2788	0.38%
	Total			28.2968	38.14%
DAY	Sample #3	Temp.	Density	Fat Extracted (g)	% of Fat Extracted
7	Dehydration 1	-25 C	98.8%	24.769	23.48%
14	Dehydration 2	-25 C	98.8%	17.6698	16.75%
21	Dehydration 3	-25 C	99.2%	13.0914	12.41%
	Total			55.5302	52.64%
28	Defat 1	20 C	98.3%	32.3306	30.65%
35	Defat 2	20 C	99.0%	7.519	7.13%
42	Defat 3	20 C	99.2%	0.7044	0.67%
	Total			40.554	38.45%
DAY	Sample #4	Temp.	Density	Fat Extracted (g)	% of Fat Extracted
7	Dehydration 1	-25 C	98.6%	32.1806	38.46%
14	Dehydration 2	-25 C	99.0%	14.3588	17.16%
21	Dehydration 3	-25 C	99.5%	6.2252	7.44%
	Total			52.7646	63.06%
28	Defat 1	20 C	98.7%	21.2876	25.44%
35	Defat 2	20 C	99.0%	3.6454	4.36%
42	Defat 3	20 C	99.4%	0.5924	0.71%
	Total			25.5254	30.51%

Table 1. Raw data over 42-day period of dehydration (-25º C) and defatting (20º C) stages.

Total fat extraction was measured by first weighing an empty beaker (to act as a collection vessel for the rotary evaporator yield). When 50 ml of the acetone-fat solution remained in the rotary evaporator, it was transferred to the pre-weighed beaker and placed in a fume hood to complete the evaporation of acetone from the fat. Twenty-four hours later the beaker was weighed, and

the total fat extraction calculated (difference between beaker with fat weight and empty beaker weight). The weight of fat extracted was divided by the initial weight of the sample to give a percentage of fat extracted.

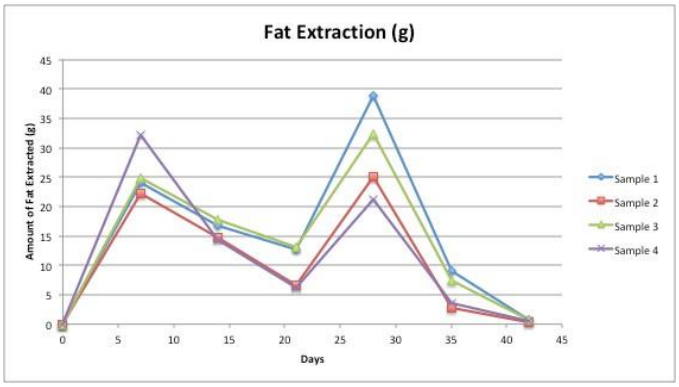

Figure 1. Fat extracted (g) every 7 days over the 42-day period from the 4 samples

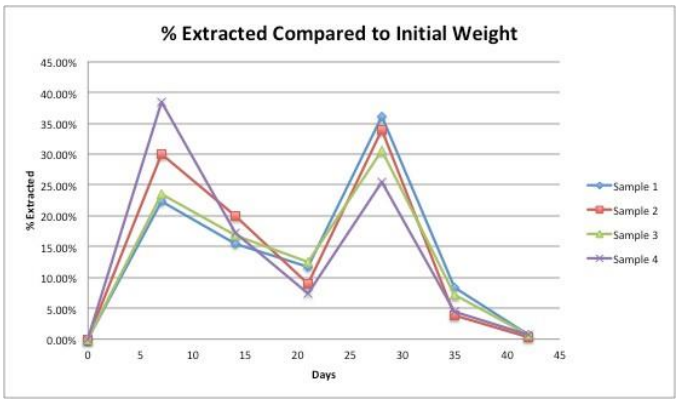

Figure 2. Percentage of extracted fat compared to initial sample weight (%). The weight of fat extracted from Figure 1 divided by the samples initial weight. To yield representable results so that they can be compared to one another without bias.

Results

The initial weight of each sample was: 107.1g (sample 1), 74.2g (sample 2), 105.4g (sample 3), and 83.7g (sample 4). Table 1 lists the results of fat extraction occurring over the 21-day dehydration period and the 21-day defatting period. From the 'Fat extracted data', lines were plotted and are shown in Figure 1. In order to compare the samples against each other, the weight of fat extracted was divided by the initial weight of the sample to give a percentage (represented by "% of Fat Extracted" column in Table 1).

Fat extraction ranged from 0.38% to 38.4% and was dependent on time. This was graphed as a line (Fig. 2). The four points of each time interval (day 7: 28.5%; day 14: 17.3%; day 21: 10.1%; day 28: 31.5%; day 35: 5.9%; day 42: 0.6%) were averaged and slopes were calculated to represent rate of change (Table 2). These K-values were used to compile a line of best fit representing all four fat sample's extraction rate over time (Fig. 3). During the dehydration phase there was 17.9% more fat extraction when compared to defatting data (56.0% extracted by weight versus 38.1%, respectively).

K-values	
Days	Slope
0-7	4.0789
7-14	-1.6014
14-21	-1.0296
21-28	3.0579
28-35	-3.6575
35-42	-0.7586

Table 2. K-values of average fat extraction rates. Slopes for the given interval periods average out from each of the four samples and are plotted in Figure 3.

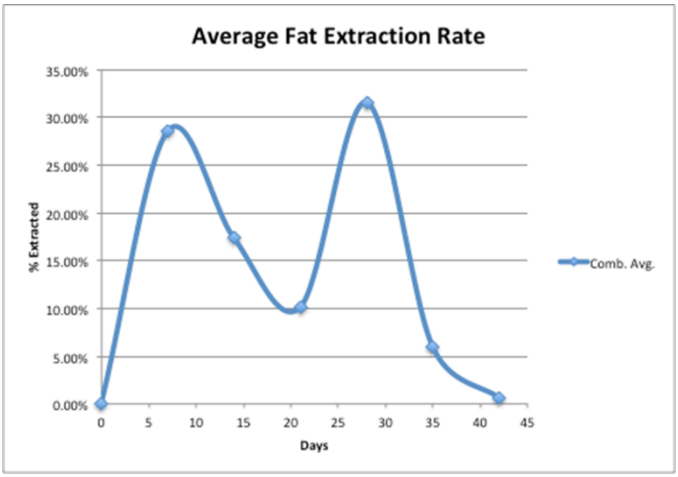

Figure 3. Average fat extraction rate (%). Averages for each time interval were calculated from table 1 and are as follows: day 7: 28.5 %; day 14: 17.3 %; day 21: 10.1 %; day 28: 31.5 %; day 35: 5.9 %; day 42: 0.6 %. The slope of this line is represented in Table 2.

Figure 4 displays acetone purity levels that were measured after each 7-day period. It was used as a tool to confirm how far along in the dehydration process each sample was (i.e. acetone density around 96% would indicate that a significant amount of water is currently being extracted from the tissue, and additional acetone changes should be performed).

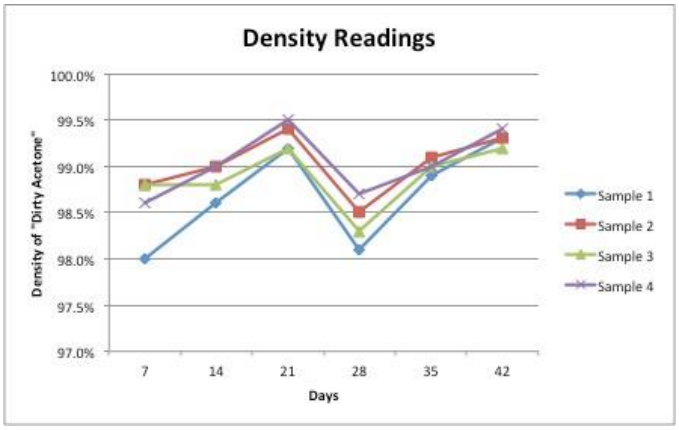

Figure 4. Density readings (%). Taken using an acetonometer calibrated at 20º C. Represent acetone purity levels.

Discussion

Based on these data, several points can be concluded, the first of which was that dehydration at -25º C and defatting at room temperature were both nearly complete after three weeks. This was verified by acetonometer readings of over 99% at the end of 21 days and 42 days. Secondly, the 21-day period marking the dehydration stage removed a little more fat than the actual 21-day degreasing phase, which disproves the assumption that the defatting phase constitutes the majority of the fat loss. The dehydrating phase showed more defatting than the actual defatting phase (56.0% versus 38.1%, respectively).

This is supported by the fact that degreasing takes seven fewer days to reach <10% fat extracted during defatting versus dehydration. It is also reinforced by basic analysis in Table 1, which shows greater fat extraction (in weight/%) during the first 21 days in comparison to the following 21 days. It is very well known that fat extraction by acetone at room temperature behaves differently than -25º C acetone (von Hagens, 1986). It confirms that when the dehydration phase is completed, the defatting phase commenced by bringing the acetone up to room temperature, because there is more fat to be extracted.

One could argue that the lower amount of fat left to be removed during the defatting phase is the consequence of the higher rate that took place during the dehydration phase. We found that the time for the defatting phase should be adjusted according to these findings in order to optimize aesthetic results of the plastinated specimen, and effectively save time/money. The degree to which dehydration and defatting should be lengthened or shortened will depend on the sample being treated. The fat contents in our samples were of one kind and uniform and are not representative of all different fat types found in all specimens to be plastinated. Our samples contained predominately one cell type (white adipose tissue). Would other type of fat degrease differently?

We can safely say that our goal for quantifying a “successful extraction of fat” was achieved and supported by density readings along with the change in slope of fat extraction. It is true that fat extraction can be proven quantitatively. Currently, discoloration of the acetone bath is the determining factor that dictates when and how often the defatting acetone baths should be changed. This process is imperfect and time consuming and may lead to a poorly defatted sample. Using the rotary evaporator, allows fat to be separated from the volatile intermedium, thus leading to a measurement value that can be compared to where the sample is in the procedure. By collecting measurements on fat extraction during various times in the plastination process, this study provides evidence of acetone’s efficiency as a defatting agent, not only during the defatting phase but also during dehydration at -25º C. This quantitative measurement of fat offers value to the plastination procedure because it will point out when the fat extraction is maximum or complete, saving time and money and guaranteeing optimal aesthetic quality of the plastinated tissues.

Acknowledgements

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind’s overall knowledge that can then improve patient care. Therefore, these donors and their families deserve our highest gratitude. Our special thanks to Dianne Durliat , Elizabeth Dalzel and the

Anatomical Donation Program of the College of Medicine of the University of Toledo.

References

Avram AS, Avram MM, James WD. 2005: Subcutaneous Fat in Normal and Diseased States. Journal of the American Academy of Dermatology, vol. 53 (4): 671-683.

DeJong K, Henry RW. 2007: Silicone Plastination of Biological Tissue Cold-Temperature Technique Biodur S10/S15 Technique and Products. Journal of the International Society for Plastination, 22: 2-14.

Henry RW, 2007: Silicone Plastination of Biological Tissue: Cold-Temperature Technique North Carolina Technique and Products. Journal of the International Society for Plastination, 22:15-19.

O'Brien JS, Sampson EL. 1965: Lipid Composition of the Normal Human Brain: Gray Matter, White Matter, and Myelin. Journal of Lipid Research, 6: 537-544.

Verma SK, Nagashima K, Yaligar J, Michael N, Lee SS, Xianfeng T, Gopalan V, Sadananthan SA, Anantharaj R, Velan SS. 2016: Differentiating Brown and White Adipose Tissues by High-Resolution Diffusion NMR Spectroscopy. Journal of Lipid Research, 58 (1): 289-298.

Von Hagens G. Heidelberg Plastination Folder 1985: Collection of All Technical Leaflets for Plastination. Anatomisches Institut I Universität Heidelberg, 1985.

Von Hagens G. Heidelberg Plastination Folder 1986: Collection of All Technical Leaflets for Plastination, Anatomisches Institut I Universitat Heidelberg, 2nd English Edition.

Wu J, Boström P, Sparks LM et al., 2012: Beige Adipocytes are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. Cell, 150 (2): 366-376.

Young G, Nippgen F, Titterbrandt S, Cooney MJ. 2010: Lipid Extraction from Biomass Using Co-Solvent Mixtures of Ionic Liquids and Polar Covalent Molecules. Separation and Purification Technology, 72 (15): 118-121.