Release Notes 2021-12-04 : I had mentioned on LinkedIn a few times that I thought pantothenate may be a decent emulsifier based on its structure and observed effects when mixing food ingreients. It appears to help wetting tryptophan and I finally did some more controlled at-home tests. This is not scientifically profound, you sometimes see panothtenate labelled as an emulsifier, but it may not be well understood that vitamins have physical properties. It solves an immediate problem - trying to get tryptophan to wet in Diet Coke or coffee or dog foods- and motivates a larger inquiry. **This is a draft and has not been peer reviewed or completely proof read but released in some state where it seems worthwhile given time or other constraints. For information only, not for use for any particular purpose see fuller disclaimers in the text. Caveat Emptor.**

Wetting Tryptophan With Calcium Pantothenate

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(Dated: December 4, 2021)

All chemicals have physical or other properties that may be forgotten with some label such as "vitamin." In the case of hydrophobic nutrients, incidental mixtures with surfactants or emulsifiers may change success or outcome of a formulation. Tryptophan is an excellent example of a hydrophobic nutrient that may be manipulated by other components of a formulation. This work explores pantothenate, without controls or comparators, as a vitamin that can enhance wetting of tryptophan while motivating larger issues in nutrient behaviors both *in vitro* and *in vivo*.

1. INTRODUCTION

Hydrophobic nutrients can be difficult to integrate with formulations intended for consumption. Personal observations with tryptophan suggest it is difficult to even wet in common beverages like water, coffee, or Diet Coke at reasonable concentrations (1/4 tsp/cup or so). It was further observed (unpublished observation) that it would segregate as a powder into the extra virgin olive oil (EVOO) component of an EVOO:water mixture although no attempt was made to determine dissolved concentrations. This behavior creates a concern about absorption in the digestive tract but an emulsifier or wetting agent may avoid these problems.

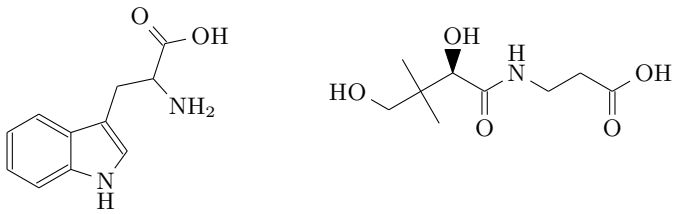

FIG. 1: Tryptophan (left) and pantothenate (right) from NCBI Pubchem.

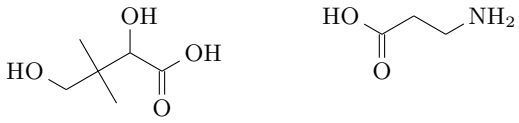

FIG. 2: Typical breakdown products of pantothenic acid : pantoic acid (left) and $\beta-$alanine (right) from NCBI Pubchem.

Exploration of the wetting and dissolution of tryptophan may help elucidate biologically important activities. Tryptophan is well known to confer important properties to biological proteins due to its solvent preferences [7] but less obvious attributes may not be fully appreciated. Tryptophan was observed to help yeast survive SDS challenge [10] and there may be more interesting properties to be discovered with simple investigations. Solubility data for proteinogenic amino acids is still a research topic. A recent work showing solubility in a water:ethanol mix

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demonstrated tryptophan to have unusual behavior with a significant peak at about .3 ethanol molar fraction as opposed to flat or decreasing curves for most others (only tyrosine had similar behavior) [5] (or the related thesis [4]) .

While commonly considered as just a water soluble vitamin, calcium pantothenate is sometimes labelled as an emulsifier [1]. The pantothenate contains one amide nitrogen, perhaps allowing it to be called an amide containing surfactant [11] , and a neopentyl glycol from the pantoic acid component which may contribute some oil solubility. Hydration of pantothenate has recently been explored with quantum mechanical simulations [6] although little work on interactions with oils was found.

Both components are unstable and potentially able to react under relevant conditions. Tryptophan is subject to photooxidation and pantothenate is well known to breakdown into pantoic acid and $\beta-$ alanine under pH extremes [9] .

This work largely just illustrates that the combination appears to mix the water and EVOO phases but hopefully motivates larger interest and consideration of the problems. An unstable emulsifier would not normally be a "first choice" but it may be suitable at point of use and unintentional usage may be an important confounding factor in performance of related products.

2. METHODS

2ml of well water (high mineral content) and old extra virgin olive oil were put into 10ml graduated cylinder. Tryptophan [3] and calcium pantothenate [2] were added sequentially. No agitation was performed except light shaking until after the pantothenate was added at which point the cylinder was vigorously shaken to mix the components and allowed to settle. At various steps, the cylinder was illuminated with a white LED flashlight (and varying amounts of ambient light) or a red laser pointer and photographed with a cell phone camera. The laser pointer illuminated the sample at a right angle from the observation direction hopefully allowing for 90 degree scattering visualization. Presumably micelles and undissolved matter can contribute to the observed scatter. The polarization and orientation of the light source are unknown. Similarly, details of the imager and signal processing chain are not known but potentially important as the areas of intense red laser illumination appear white only in the photos but appeared red during visual observation.

Often "limitations of study" are discussed in the discussion section but this is so limited as to deserve mention early on. Note that for a reasonable study, negative and positive controls should have been included to compare pantothenate to known emulsifiers and inert components. This work is just to demonstrate that pantothenate can help wet tryptophan although chemical modifications can not be ruled out. Response curves, varying amounts of pantothenate without tryptophan for example, could also be included .

3. RESULTS

The results are presented in chronological order as ingredients were added. An overview composite photograph with all results is show in Fig. 3 but each row is shown expanded in sequence later. Combinations of red laser pointer light and white light from the flashlight at various angles are responsible for the different color distributions except that the white color in areas of intense laser pointer red light was not observed by eye and likely reflects imager saturation or overload. It is not known how the imaging system deals with overflow or color. In all cases illumination was from the left or top with the laser pointer and right angle scattering is nominally observed .

FIG. 3: All the results : top row is original 50:50 water-oil, next row includes addition of tryptopham, third row includes calcium pantothenate, finally the bottom is after settling over night.

Initially only water and oil were in the cylinder. The water was added first and never shaken. The first image on the left illustrates laser pointer illumination from the top documenting baseline scattering from the EVOO and well water below. Some scattering at interfaces is observed. The second or middle image shows the gold color of the oil and clear water below (illumination from the left). The third image shows combined illumination for better context.

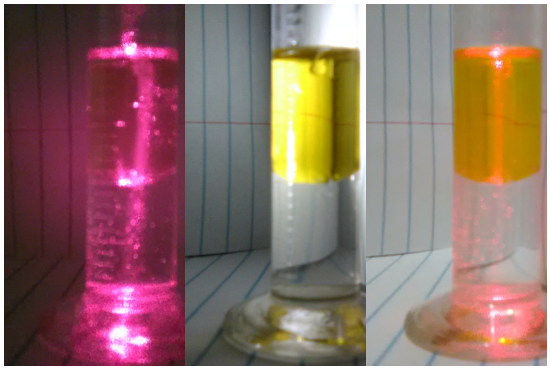

FIG. 4: Oil and water with differing illumination. The left or first is illuminated with a red laser pointe from the top. Note the straight dim line of scattered light. The second one is just white light, mostly reflected, and the third combines white and laser light.

After adding the tryptophan, again with only very mild agitation, 8 photographs were obtained, Fig. 5, in an attempt to illustrate the tryptophan sinking to the bottom of the oil layer. The first and second of the four laser pointer photos (right) shows illumination from the top and scattering at the interaces. The latter two show horizontal illumination in the water layer(left) and oil layer (right) with slightly more scatter.

FIG. 5: Addition of Trp did nothing except create white layer at bottom of oil . The added tryptophan quickly wetted on the oil surface and sank. It is unknown how much dissolved into either phase.

After adding the calcium pantothenate and shaking to disrupt oil-water interface, , mixing was observed as shown in Fig. 6 . Note that no vigorous shaking or agitation was performed in prior steps as would occur in a well controlled test but see comments in Methods and Introduction. The left photo with white light only demonstrates largely cloudy mixture with only a small clear column at the bottom. The scatter is illustrated with the laser pointer showing some visible scatter in the water but significantly more in the yellow cloudy layer. Turning off the white light does not help much.

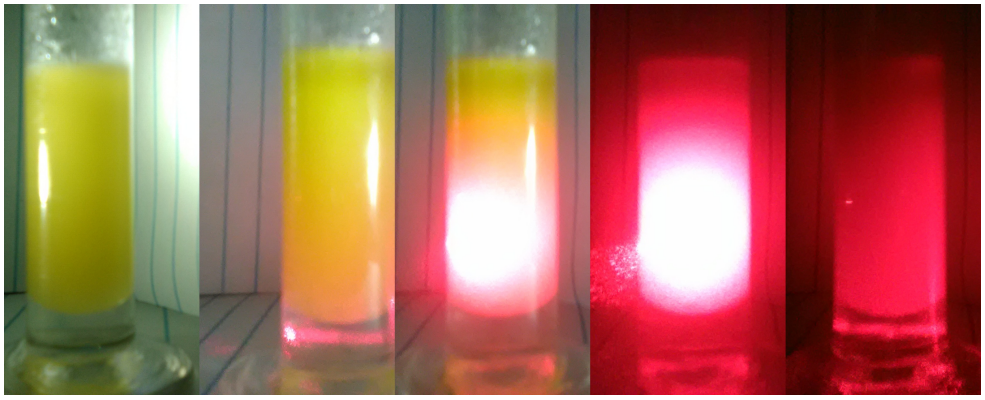

FIG. 6: Combining with calcium pantothenate and shaking created more scatter and mixing.

The apparent emulsion remained largely stable overnight as shown in Fig. 7 although three layers were clearly observed in white light. The bottom clear layer boundary retained a meniscus while a flat layer separates the two yellow cloudy regions. With laser illumination from the left, intense scattering could be observed and leaving the white light on provided better visual context. First, illumination was applied from the left illustrating beam spreading. The fifth photo shows illumination from the top that appears to stop at the layer boundary. Illuminating the bottom yellow layer from the left suggests no light gets to the top layer and any light that goes into the clear layer below is apparently not scattering toward the camera.

FIG. 7: Little change was observed over night although the oil contents settled into two layers. Possible contributors to scatter are micelles and suspended solids.

4. DISCUSSION

The calcium pantothenate and tryptophan combination apparently emulsifies the water-oil mixture inline with expectations after observing the panothenate facilitate trytophan wetting . This was not intended to compare to other possible wetting agents or emulsifiers although many simple control tests and extensions suggest themselves. Cleaner liquids such as DI water and a less complicated oil phase would help as would agitation at more steps. Positive and negative controls instead of the pantothenate would create more of an indication of panotothenate's uniqueness in this role. Many other putative emulsifiers exist.

Pantothenate is known to be unstable and various techniques have been investigated to improve this [8]. There can be no assurance that chemical or photochemical reactions did not occur with the impure liquids of old olive oil and well water. Breakdown products are typically pantoic acid and $\beta-$alanine. Both products are soluble in water and in varying other solvents.

5. CONCLUSIONS

Calcium pantothenate allows water to wet trytophan and to form a water and oil emulsion. It is not known how the tryptophan is distributed but it does not seem to be segregated to a pure oil phase. This observation may relate to the immediate problem of getting tryptophan to wet in diet coke or coffee and this simple system may be useful for understanding more obscure behaviors of tryptophan in biological systems.

6. SUPPLEMENTAL INFORMATION

6.1. Computer Code

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making the composite images,

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cat origs.tex | while read
do

x=‘echo "$REPLY" | awk ’{print "convert "$1" -crop 2000x600+"$3"+"$4" -rotate 90 "$2".jpg"}’‘
echo $x
$x
done
#convert keep/trpetc/overnight/IMG_20211130_050559.jpg -crop 1000x600+0+600 -rotate 90 final_vis.jpg


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    .jpg cropped11.jpg trponly.jpg
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    .jpg cropped9.jpg trponly.jpg
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    jpg
convert -append orgingals.jpg trponly.jpg combined.jpg onight.jpg composite.jpg
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Acknowledgments

1. Pubmed eutils facilities and the basic research it provides.
2. Free software including Linux, R, LaTex etc.
3. Thanks everyone who contributed incidental support.

Appendix A: Statement of Conflicts

No specific funding was used in this effort and there are no relationships with others that could create a conflict of interest. I would like to develop these ideas further and have obvious bias towards making them appear successful. Barbara Cade, the dog owner, has worked in the pet food industry but this does not likely create a conflict. We have no interest in the makers of any of the products named in this work.

Appendix B: About the Authors and Facility

This work was performed at a dog rescue run by Barbara Cade and housed in rural Georgia. The author of this report ,Mike Marchywka, has a background in electrical engineering and has done extensive research using free online literature sources. I hope to find additional people interested in critically examining the results and verify that they can be reproduced effectively to treat other dogs.

Appendix C: Symbols, Abbreviations and Colloquialisms

TERM	definition and meaning
EVOO	Extra Virgin Olive Oil
SDS	Sodium Dodecyl Sulfate

Appendix D: General caveats and disclaimer

This document was created in the hope it will be interesting to someone including me by providing information about some topic that may include personal experience or a literature review or description of a speculative theory or idea. There is no assurance that the content of this work will be useful for any paricular purpose.

All statements in this document were true to the best of my knowledge at the time they were made and every attempt is made to assure they are not misleading or confusing. However, information provided by others and observations that can be manipulated by unknown causes ("gaslighting") may be misleading. Any use of this information should be preceded by validation including replication where feasible. Errors may enter into the final work at every step from conception and research to final editing.

Documents labelled "NOTES" or "not public" contain substantial informal or speculative content that may be terse and poorly edited or even sarcastic or profane. Documents labelled as "public" have generally been edited to be more coherent but probably have not been reviewed or proof read.

Generally non-public documents are labelled as such to avoid confusion and embarassment and should be read with that understanding.

Appendix E: Citing this as a tech report or white paper

Note: This is mostly manually entered and not assured to be error free.
This is tech report MJM-2021-014.

Version	Date	Comments
0.01	2021-11-30	Create from empty.tex template
-	December 4, 2021	version 0.80 MJM-2021-014
0.8	2021-12-04	Draft Note form, time constraint
1.0	20xx-xx-xx	First revision for distribution

Released versions,
build script needs to include empty releases.tex

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.80 table	2021-12-04	https://www.academia.edu/s/88b01398d4
.80 table	2021-12-04	https://www.linkedin.com/posts/marchywka_wetting-tryptophan-with-pantothenate-activity-687307460454

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Supporting files. Note that some dates,sizes, and md5's will change as this is rebuilt.
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