Enzymatic Extract Fractionation of (*Capsicum Annuum*) Hot Chili Pepper through Supercritical Carbon Dioxide (SC-CO_2)

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ABSTRACT

Capsicum annuum or hot chili is very common in the Philippines as spice, many research studies were already conducted and many extraction methods were already done but no study fractionated the enzymatic extract of chili pepper. In this study, an enzymatic hot chili extract from DA-PIU was fractionated using the supercritical carbon dioxide to obtain different oil extracts in three different parameters, 10Megapascal (MPa), 20Megapascal (MPa) and 30Megapascal (MPa) at constant temperature at 40° Celsius. The highest oil yield is at 20MPa with an average of 3.15% followed by 10MPa with 2.05% and 30MPa at 1.02%, all extracts are in triplicates. 10MPa oil sample was subjected to gas chromatography- mass spectrometry (GC-MS) analysis and found twenty eight compounds in which palmitic acid and capsaicin were the major compounds. For fatty acid profiling, pure crude enzymatic, 20MPa and 30MPa sample was subjected to gas chromatography (GC) at DOST-ITDI and was compared. Essential fatty acid linoleic (C18:2) was highest in pure crude enzymatic, while lauric (C12) was consistently highest for 20MPa and 30MPa in terms of weight by weight. Comparing the fatty acid profile, two fatty acid was not found in pure crude and present in 20MPa and 30MPa namely undecanoic (C11) and tridacanoic (C13), while one fatty acid was found in pure crude and was not present in 20 and 30 MPa namely cis-11, 14-eicosadienoic (C20:2). Cis 13, 16- docosadienoic (C22:2) AND CIS- 4, 7, 10, 13, 16, 19- docosahexanoic (C22:6) were only found in 30MPa sample only.

Keywords**:** supercritical carbon dioxide fractionation, enzymatic, gas chromatography, mass spectrometry fatty acid profiling

INTRODUCTION

Red Hot Chili Pepper

Capsicum annuum or chili peppers are widely accepted both for food and flavoring, especially in an agricultural country such as the Philippines. This plant possesses a pleasant aroma which enhances the flavor of many fresh and cooked foods (1). One study experimented a variety of this plant from Chile, Hungary and Turkey which was rehydrated and then evaluated by descriptive and hedonic panels, the volatile compounds were analyzed by gas chromatography using flame ionization detection, mass spectrometry and sniffing port detection, forty six compound were identified, 12 of which possessed odors: 2-methylpropanal, 2 and 3-methylbutanal, 2,3-butadione, 1-penten-3-one, hexanal, heptanal, beta-ocimene, trans-3-hepten-2-one, dimethyltrisulphide, 2-methoxy-3-isobutylpyrazine and beta-cyclocitral which has a fruity odor. Using steam distillation and conventional capillary gas-liquid chromatographic separation major components identified was 2-methoxy-3-isobutylpyrazine, trans-beta-ocimene, limonene, methyl salicylate, linalool, nona-trans, cis-2,6-dienal, deca-trans, trans-2,4-dienal and hex-cis-3-enol, 2-methoxy-3-isobutylpyrazine was found to possess an extremely potent odor(2-3). Dynamic headspace gas chromatography, mass spectrometry and sniffing port detection were used to analyze the volatile compounds, the different samples obtained had several odor compounds in common: 2,3-butanedione, 1-penten-3-one, hexanal, 3-carene, (Z)-bet-ocimene, octanal and 2-isobutyl-3-methoxypyrazane (2-3).

Supercritical Carbon dioxide Fractionation

Supercritical Carbon dioxide (SC-CO_2) fractionation is very useful especially when conducting experiments using natural product crude extracts because SC-CO_2 can be performed at lower temperatures that preserve original composition and properties of extracts (4).

Another advantage of this extraction is that CO_2 is non- toxic, non- flammable, non-corrosive, non- explosive, cheap and readily available in bulk quantities with a high degree of purity. CO_2 also has low critical temperature and pressure which make it ideal solvent for natural products since they thus not undergo thermal degradation during the fractionation process (5).

The supercritical separation technology using carbon dioxide as solvent makes possible not only the design of environment friendly process, but also the processing of biological materials and the possibility of obtaining products free of solvent residuals and using the properties of supercritical carbon dioxide we can fractionate the enzymatic crude extract from the material to be tested.

Fractionated Products and the Industry

Supercritical fluid extraction demonstrates the ability to purify several types of surfactants which is important for pharma solutions. Since common pharma applications has been processed with traditional technologies. SC-CO_2 separates materials on the basis of solubility not vapor pressure and thus it can purify heat sensitive materials that cannot be processed by distillation (6). SC-CO_2 method was used to produce solvent free extract and will be concentrated in the active components of the oil (7).

Fractionation is a term used in the method of controlling the parameter particularly in the pressure. Experimenting with different pressures (10MPa, 20MPa, 30MPa) can extract different compounds, usually samples at 10MPa resulted to low volatile compounds that is commonly used in aromatic perfume formulas if further isolation will be done. Samples at 20MPa and 30MPa are usually tested for fatty component which can be used for different industry which depends on the percentage of specific fatty acid content. Some articles cited that selected medical components are currently being processed with the supercritical fluids to remove undesired species or what we call as purification process.

In this study, test of combined methods of enzymatic extraction and SC-CO_2 for different fatty acid profile and identification of compounds from 10MPa fractions are the limitations.

METHODOLOGY

Enzymatic Extract

The enzymatic extract was obtained from the byproduct researches of Department of Agriculture- Biotech Agricultural Research (DA-BAR), Philippines. Mixture of enzymes was carried out to the plant samples to obtain the enzymatic extracts.

SC-CO_2 Fractionation

The Akico Supercritical Carbon dioxide (SC-CO_2) set up was used for fractionation. This apparatus can operate at temperatures between 40 and 80 ^{o}C and pressures up to 30.25MPa. Fractionation experiments were conducted using pure CO_2; the same sample was used for 10MPa, 20MPa and 30MPa at constant temperature of 40^{o}C with fractionation time for two hours for each parameter. The fraction was collected using 10mL glass tubes in the sample collection which will be weighed previously by an analytical balance and will be reweighed again after the sample collection and calculated for the oil yield.

Sample Analysis

For the sample analysis, the 10Mpa extracts was subjected to gas chromatography-mass spectrometry (GC-MS Shimadzu QP2010) with Supelco SPB-5 fused capillary column, 280^{o}C injector port temperature ad helium as gas carrier for identification of possible compounds present in the sample. The 20MPa and 30MPa extracts were subjected to the fatty acid profiling by gas chromatography (GC Shimadzu 2010) with SP-2560 capillary column using the method by Christie with operating temperature of 140 degree Celsius and further increase to 240 degree Celsius.

RESULTS AND DISCUSSIONS

Oil Yield

Triplicate sample was obtained from the fractionation using supercritical carbon dioxide. Shown in figure 1 the percent oil yield of the enzymatic hot chili pepper extracts, 20 Mega pascal (MPa) extracts has the highest oil yield at 3.15% followed by 10MPa with 2.05% and 30MPa with 1.02%.

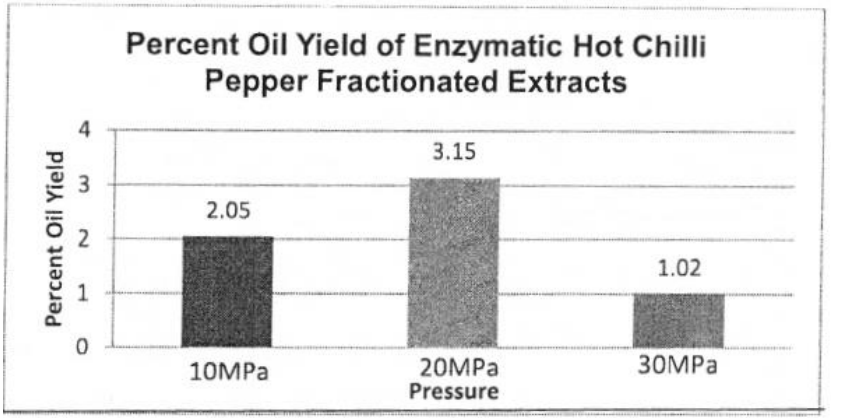

Figure 1. Percent Oil Yield of Enzymatic Hot Chili Pepper fractionated Extracts

10MPa Fractionation of Enzymatic Hot Chili Pepper Extract

10MPa fraction is an essential oil of enzymatic hot chili pepper extract as shown in figure 2 identified by gas chromatography- mass spectroscopy. There are twenty eight compounds found in which palmitic acid and capsaicin were the major compounds.

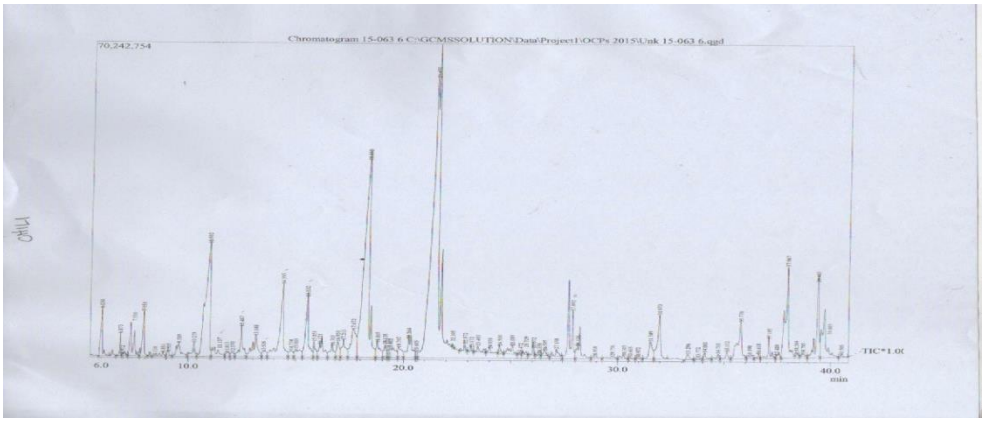

Figure 2. GC Chromatogram of Enzymatic Hoot Chili Pepper 10MPa Extract

Table 1 shows the compound found in 10MPa Fraction of Enzymatic Hot Chili Pepper

Table 1. Compound found in 10MPa Fraction of Enzymatic Hot Chili Pepper

Retention Time/ Peak	Compound Name
6.972	4-ehtyl-2-methoxy-phenol
8.138	(2-cyclohexenyl-3-[1-methyl-3-oxo-1-butenyl]-2,4,4-trimethyl) ester
12.487	Cinnamyl tiglate
13.148	Stearophenone
13.508	Myrictic Acid
15.532	Pentadecanoic Acid
15.851	9-Hexadecenoic Acid
16.705	Chlorooctadecane
17.213	n-tridecanoic Acid
18.332	Palmitic Acid
19.222	Eicosanoic Acid
20.264	9,12-Octadecadienoic Acid
21.470	linoleoyl chloride
22.872	Lauric Acid
23.172	N-hexylacetamide
23.483	Nerolidyl acetate
25.472	Argyrophilic Acid
25.729	1,6,10-Dodecatriene-3-Carboxylic Acid
27.892	Capsaicin
28.135	Hexanoic Acid
30.605	3.3.3-trifluoro-2-hydroxy-propionic acid-2-isopropyl-5-methyl cyclohexyl ester
31.549	Octanoic Acid
34.081	3-beta-acetoxy-5-alpha-androstan-7-one
35.132	Nerolidyl propionate
35.776	1-mono laurin
37.105	N_2O_6-Dimethyl guanine
37.967	Octadecanoic Acid
39.605	Hexadecanoic Acid

Fatty Acid Profiling of Pure Crude Enzymatic Extract, 20MPa and 30MPa Fractions of Hot Chili Pepper

Table 2 shows the fatty acid profiling of different extracts from enzymatic hot chili pepper (C18:2), essential short chain polyunsaturated fatty acid is highest in terms of weight by weight for pure crude, while lauric (c12) was consistently highest for 20MPa and 30MPa fractions in terms of weight by weight. There are twenty one fatty acid found in pure crude enzymatic, twenty two fatty acid found in 20MPa fraction and twenty four fatty acid found in 30MPa fractionate. Comparing the fatty acid profile, two fatty acid was not found in pure crude and was found in pure crude and was not present in 20 and 30MPa namely cis- 11,14-eicosadienoic (C20:2). Cis 13, 16-docosadienoic (C22:2) and cis-4, 7, 10, 13, 16, 19-docosahexanoic (C22:6) were only found in 30MPa fraction only. Short chain polyunsaturated fatty acid linoleic (C18:2) and linolenic (C18:3) with long chain polyunsaturated fatty acid y-linolenic (C18:3n6) were consistently found in pure crude enzymatic, 20MPa and 30MPa fractions respectively.

Table 2. Fatty Acid Profile (%w/w) of Pure Crude Enzymatic, 20MPa and 30MPa Fractions of Hot Chili Pepper

Fatty Acid	Pure Crude Enzymatic	20MPa	30MPa
Caproic (C6)	1.15	0.654	0.92
Caprylic (C8)	4.42	7.75	7.01
Capric (C10)	4.35	7.76	7.08
Undecanoic (C11)	Not Detected	0.0494	0.0428
Lauric (C12)	20.8	32.8	31.4
Tridecanoic (C13)	Not Detected	0.0624	0.0694
Myristic (C14)	6.23	7.92	8.02
Pentadecanoic (C15)	0.172	0.211	0.189
Cis-10- Pentadecenoic (15:1)	0.424	0.611	0.519
Palmitic (C16)	6.64	4.68	4.96
Palmitoleic (C16:1)	2.48	2.44	2.34
Heptadecanoic (C17)	0.604	0.528	0.554
Stearic (C18)	3.34	2.22	2.45
Oleic (C18:1)	6.44	3.76	4.08
Linoleic (C18:2)	28.6	16.9	18.4
y-Linolenic (C18:3n6)	0.353	0.189	0.203
Linolenic (C18:3)	13.0	11.0	11.0
Erucic (C22:1n9)	1.00	0.672	1.09
Cis-11,14- eicosadienoic (C20:2)	0.0560	Not Detected	Not Detected
Behenic (C22)	0.0656	0.0256	0.0304
Erucic (C22:1n9)	0.241	0.112	0.0517
Cis-13, 16- Docosadienoic (C22:2)	Not Detected	Not Detected	0.0334
Lignoceric (C24)	0.153	0.0680	0.246
Cis-5,8,11,14,17- Eicosapentaenoic (C20:5)	0.159	0.0456	0.0775
Cis-4,7,10,13,16,19- Docosahexaenoic (C22:6)	Not Detected	Not Detected	0.147
Number of Short Chain Polyunsaturated FA	2	2	2
Number of Long Chain Polyunsaturated FA	1	1	1

Comparison of Compounds from Fractionated SC-CO_2 Enzymatic Hot Chili Pepper vs. Non- Enzymatic Extraction

Table 3. Comparison of Compounds from Fractionated SC-CO_2 Enzymatic Hot Chili Pepper vs. Non- Enzymatic Extraction

Fractionated SC-CO2 Enzymatic Hot Chili Pepper Compounds	Non Enzymatic Extraction (Steam Distillation)*
4-ethyl-2-methoxy-phenol	2-methoxy-3isobutylpyrazine
(2-cyclohexenyl-3-[1-methyl-3-oxox-1-butenyl]-2,4,4-trimethyl) ester	trans-beta-ocimene
Cinnamyl tiglate	Limonene
Stearophenone	Methyl salicylate
Myristic Acid	linalool
Chlorooctadecane	Nona-trans,cis-2,6-dienal
9-Hexadecenoic Acid	Deca-trans,trans-2,4-dienal
n-tridecanoic Acid	Hex-cis-3-enol,2-methoxy-3isobutylpyrazine
Palmitic Acid	
Eicosanoic Acid	
9,12-Octadecadienoic Acid	
Linoleoyl chloride	
Lauric Acid	
N-hexylacetamide	
Nerolidyl acetate	
Argyrophilic Acid	
1,6,10-Dodecatriene-3-Carboxylic Acid	
Capsaicin	
Hexanoic Acid	
3,3,3-trifluoro-2-hydroxy-propionic acid-2-isopropyl-5-methyl cyclohexyl ester	
Octanoic Acid	
3-beta-acetoxy-5-alpha-androstan-7-one	
Nerolidyl propionate	
1-mono laurin	
N_2O_6-Dimethyl guanine	
Hexadecanoic Acid	
Octadecanoic Acid	

*Buttery Et al & Luning Et al., 1994

Take note that the data may differ because of the type and the quality of the sample. Definite conclusions can be done once the same sample use for enzymatic extract will be extracted without the treatment of enzyme. For this comparison, there are a lot of compounds found in enzymatic SC-CO_2 fractionation compared to non-enzymatic steam distillation extraction.

SUMMARY AND CONCLUSIONS

Oil Yield and Fatty Acid Profiling

For enzymatic Hot Chili Pepper fractionation, the highest yield is at 20MPa. Palmitic and Capsaicin were the major compounds found in 10MPa fraction. For fatty acid profiling Linoleic (C18:2) was highest in terms of (%w/w) in pure crude extract, while Lauric (C12) is highest in terms of (%w/w) in 20 and 30MPa fractionated extracts.

Importance of Fatty Acids

Fatty acids (FAs) are compounds containing a long hydrocarbon chain and a terminal carboxylic group. Two major physiological roles of fatty acids are one, building blocks of phospholipids and glycolipids in biological membranes and two a fuel molecules. FAs chain length are defined to the number of carbon atoms from short chain FA (<6 carbon atoms) to very long chain fatty acids (>20 carbon atoms) with a certain degree of saturation, the presence or absence of double bonds. There are more than 100 different FAs in nature in which for plants and animals, the predominant are palmitic, oleic, linoleic and stearic acids. Linoleic and linolenic acids are important essential fatty acids required for growth, physiological functions and body maintenance.

The abundance of unsaturated fatty acids like linoleic and linolenic fatty acids in oil is desirable from nutritional and health points of view as unsaturated fatty acid consumptions will not lead to heart related diseases and oil rich in unsaturated fatty acids have been reported to reduce the risk of heart diseases associated with cholesterol. Please take note that cis- unsaturated fatty acid (bend in hydrocarbon chain) like linoleic acid is better than unsaturated FAs trans isomer (straight hydrocarbon chain) which the configuration is similar to that of saturated FAs which can be deposited in human adipose tissue.

Although it might be that some majority of the samples has saturated fatty acids, this does not imply that saturated fatty acids are not useful, taking saturated fatty acids at desirable amount can be beneficial. Saturated fats like myristic and lauric play key roles in immune health. Stearic, a common saturated fatty acid is commonly used in the production of pharmaceutical tablets and capsules; it is also a stable base form for deodorants, lotions and creams. This substance is also used in many food products due to its stability during storage and frying, even though it is a saturated fat, it seems to have little effect on cholesterol levels in the blood. The reason is that high proportion of stearic acid is converted to oleic acid which is monounsaturated fat. Other uses include preventing oxidization and commonly used to coat metal powders such as iron and aluminum. FAs uses are varied depending on the needs of the industry that will further develop to commercialize in a large scale basis.

Enzymatic SC-Co_2 Fractionation

According to literatures, enzymatic extractions make the fractionation of oil much easier and improved oil extraction. This is a preliminary study that characterized the fatty acid from different fraction and identifies the compounds from 10MPa fraction and oil yield was also obtained. Optimization and comparison from the same sample without enzyme treatment are the recommendations of this study.

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REFERENCES

(1) Forero,D., Quijano,CE., Pino, JA.(2008) Volatile Compounds of Chili Pepper at two Ripening Stages Flavour and Fragrance Journal.24(1), pp.25-30.

(2-3) Buttery, RG., Seifert RM., Guadagni, DG., Ling, LC. (1969) Characterization of Some Volatile Constituents of Bell Peppers. Journal of Agric. Food Chem. 17(6), pp. 1322-1327.

(4) Reverechon, E., Donsi, G., Osseo, L.S. (1997). *Modeling of Supercritical Fluid Extraction from Herbaceous Matrices*. Industrial and Engineering Chemistry Research. 37, 2721

(5) Gil, M., Bernardo, G, Casquilho, M, Mercedes, EM, Albertina, RM. (2005). *Supercritical fluid extraction of fig leaf gourd seeds oil: fatty acid Composition and extraction kinetics*. J. of Supercritical Fluids.

(6) Krukunis, V, Brunner, G, Perrut, M. (1994). *Industrial Operations with Supercritical Fluids: Current Processes and perspectives on the future, proceedings 3rd International Symposium on Supercritical Fluids. Tome 1 p. 1. Strasbourg.*

(7) Wany, A, Jha, S., Nigam KV.,Pandey, DM. (2013). *Chemical Analysis and Therapeutic uses of Citronella Oil from Cymbopogon Winterianus: A short Review.* International Journal of Advanced Research. 1(6) 504-521.

(8) Jonka, J, Fialova, L. (2002). *Fatty acids nomenclature characterization properties and utilization.* Inst. Of Medical Biochemistry.

(9) Law, M. (200). *Dietary fat and adult disease and the implications for Childhood nutrition: an Epidemiologic approach. American Journal Clinical Nutrition*. 72: 1291-1296.