

Statistical Analysis of Solvent Polarity Effects on Phytochemicals Extraction: Multiple Correspondence Analysis (MCA) Approach

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ABSTRACT

Multiple correspondence analysis (MCA) was used in this study to statistically analyze the nature of the relationship between the polarity of extraction solvents and the type of phytochemicals extracted from mushroom caps using solvents with different polarity. The caps of three mushrooms (Enoki, Buna shimeji, and Bunapi shimeji) were extracted with four solvents of varying polarities (water, methanol, acetone, and ethyl acetate). The phytochemical content of the extracts was identified using UPLC-QTOF, followed by coding of the compounds and the subsequent MCA using SPSS[®] version 22. The MCA showed a positive correlation between the extraction solvents and the phytochemicals extracted from the mushrooms. A positive correlation of $r = 0.322$ was observed between the identified phytochemicals and the solvents used during the extraction process. The pattern of the phytochemicals clustering suggested that solvent polarity differentiated the groups of phytochemicals

extractable from the mushrooms. It is, therefore, concluded that MCA is a valid statistical tool for the determination of the relationship between solvent polarity and the types of phytochemicals extracted using such solvents.

Keywords: Multiple correspondence analysis (MCA), correlation, mushroom phytochemicals, solvent polarity, statistical analysis.

1. Introduction

Several studies have investigated the role of solvent polarity on the extraction of bioactive compounds from various natural sources (see Addai *et al.* (2013), Dar *et al.* (2016), Hamid *et al.* (2016), Onbaşıli *et al.* (2015)). According to Hamid *et al.* (2016), the solubility of bioactive compounds in solvents is influenced by certain factors, such as the polarity of the extraction solvent, the degree of aromatic rings conjugation in the compounds, glycosidic bond formation, and the presence of side chains like methoxy, hydroxyl, and methyl groups. A good understanding of the role of solvent polarity during the extraction of bioactive compounds will facilitate a proper selection of the right solvent for an extraction process. Bioactive compounds are usually extracted from natural sources using different solvents of varying polarities. This is to ensure complete recovery of the targeted compounds from the source since it is believed that different components of a material can be efficiently extracted using different solvents of varying polarities (Policegoudra *et al.* (2011)). However, this method often involves the use of too many solvents which are believed to have diverse influences on the environment (Addai *et al.* (2013)). Thus, a statistical approach towards the selection of appropriate solvents for the extraction of bioactive compounds from natural sources is proposed in this paper. The use of multiple correspondence analysis (MCA) for the determination of the right solvents for the extraction of phytochemicals from the caps of three different edible mushrooms was conducted in this study. This is the first application of MCA for the determination of the relationship between solvent polarity and the types of phytochemicals extracted with such solvents. The aim of this study is to use MCA to determine the relationship between the phytochemicals extracted from the caps of the studied mushrooms and the type (polarity) of the solvents used during the extraction process.

MCA as a statistical method is developed as an extension of correspondence analysis (CA) which permits the analysis of the relationships between several categorical dependent variables. MCA can be considered a generalization of

the principal component analysis (PCA) in which variables are rather categorical instead of quantitative (Salkind (2006)). Technically, MCA is performed using standard correspondence analysis (CA) on an indicator matrix (i.e., a matrix whose entries are 0 or 1). In the MCA, the percentages of the explained variance need to be corrected, and the correspondence analysis interpretation of the interpoint distances ought to be adapted (see Salkind (2006)). Each nominal variable in the MCA consists of several binary coded levels. In this study, the phytochemicals identified in the extracts of *Flammulina velutipes* (Enoki), white *Hypsizygus tessellatus* (Bunapi shimeji), and brown *Hypsizygus tessellatus* (Buna shimeji) caps were correlated using MCA to the type of solvent (water, methanol, acetone, and ethyl acetate) used during the extraction processes. The analysis was performed using the MCA package in the Statistical Package for the Social Science (SPSS[®]) version 22 (IBM, USA) (see Addai *et al.* (2013)).

2. Materials and Methods

2.1 Extraction and identification of mushroom phytochemicals

The phytochemical contents of Enoki, Buna shimeji, and Bunapi shimeji caps were first extracted using water, methanol, acetone, and ethyl acetate. After the extraction process, the phytochemicals content of each mushroom fraction was identified using WATERS AGILENT 6560 UHPLC-IM-QTOF-MS/MS system.

2.2 Multiple correspondence analysis

As mentioned in section 1, the variables to be analyzed with MCA must be categorical rather than quantitative. The MCA's data table consists of binary columns, with only one column taking the value "1" per nominal variable (Escofier (1978)). The identified phytochemicals were first coded using an alpha-numeric pattern (C1 to C282, where C = compound and 1 to 282 = assigned number to each compound, refer to the Appendix section) before being loaded into the MCA software in Statistical Package for the Social Science (SPSS[®]) version 22 (IBM, USA). The complete list of the identified phytochemicals from the mushroom extracts and their codes for MCA analysis are as follows:

Identified phytochemicals and their codes

C1 = Flavone, **C2** = 5,8-Dihydroxy-2-(2-phenylethyl)-chromone,
C3 = 2'-Hydroxy-4',6'-Dimethoxy Dihydrochalcone,
C4 = 4'-O-Methyl-brazilin, **C5** = 3'-O-Acetyl-Hamaudol,
C6 = Corylin, **C7** = Licoflavone A,
C8 = 6-Methoxy-2-[2-(4'-methoxyphenyl) ethyl] chromone,
C9 = 7-Hydroxy-5,3',4'-trimethoxy Flavone,
C10 = Patuletin, **C11** = Divaricatol,
C12 = 2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-Methoxy-benzyl)
 chroman-4-one,
C13 = (3R,4S)-3,4-Dihydroxy-3-(3',4'-)-7-methoxy-chroman,
C14 = Sanggenon H,
C15 = 3,4',5-Trihydroxy-7-methoxy-8-Isopentenylflavone,
C16 = 3',5', β -Trihydroxy-3,4,4', α -Tetramethoxy-Xyechalcone
C17 = Sophoflavescenol, **C18** = Glabrol, **C19** = Aloeresin,
C20 = 3',4',5',5,7,8-Hexamethoxy Flavone, **C21** = Kuwanon S,
C22 = Chrysin-7-O- β -glucopyranoside, **C23** = Kuwanon D,
C24 = Kushenol F, **C25** = Kushenol T, **C26** = Flavenochromane A,
C27 = Dihydrokaempferol-5-O- β -D-glucopyranoside,
C28 = Kushenol I, **C29** = Kushenol G, **C30** = Kuraridinol,
C31 = Isoquercitrin, **C32** = Silymonin, **C33** = Kushenol K,
C34 = Nepitrin, **C35** = Hibiscetin-3-Oglucoside,
C36 = Nevadensin-7-O- β -D-glucoside, **C37** = Kushenol M,
C38 = Quercetin-3-Oglucuronide-6"-methyl ester,
C39 = Iridin, **C40** = Icariside-I, **C41** = Hibiscetin-3-Oglucoside,
C42 = Neomangiferin, **C43** = 2"-O-Rhamnosyl vitexin,
C44 = Nelumboside A, **C45** = Safflomin C,
C46 = Cyanidin-3rutinoside, **C47** = 2"-O-Acetyl-3'-O-methylrutin,
C48 = Awobanin, **C49** = Saponin PA, **C50** = Procyanidin B2 gallate,
C51 = 1-O-Galloyl-pedunculagin, **C52** = Cnidimol E, **C53** = Episappanol,
C54 = 6-Methoxy-2-[2-(3'-methoxyphenyl)-ethyl]-chromone,
C55 = 6-Hydroxy-2-[2-(3'-methoxy-4'-hydroxy-phenyl) ethyl]-chromone,
C56 = Leucodelphinidin, **C57** = Corylifolinin, **C58** = Licochalcone A,
C59 = Morachalcone A, **C60** = Isoxanthohumol,
C61 = Leachianone G, **C62** = Tangeritin, **C63** = Kushenol A,
C64 = Pinnatifinoside A, **C65** = Cyclomorusin,
C66 = Isomangiferin, **C67** = Quercetin 3-O-xyloside,
C68 = Sanggenon A, **C69** = Sec-Oglucosyl-Hamaudol,
C70 = Kushenol C, **C71** = Astilbin, **C72** = Cinchonain Ib,
C73 = Methyl kushenol C, **C74** = Taxifolin-3-O-glucoside,
C75 = Pelargonidin 3-Glucoside, **C76** = Sanggenon K,
C77 = Kosamol A, **C78** = Mahuannin D, **C79** = Viscumneoside II,

C80 = Isoaloeserin A, **C81** = DO 21, **C82** = Procyanidin A2,
C83 = Kushenol O, **C84** = Kaempferitrin,
C84 = Kaempferide-3-O- α -Lrhamn- α -Lrhamnoside,
C86 = Buddlenoid A,
C87 = Hispidulin 7-(6-E-pcouma-royl- β -Dgluco-pyranoside),
88 = Pectolarin, **C89** = Shegansu A, **C90** = Typhaneoside,
C91 = p-Tolualdehyde, **C92** = Syringaldehyde,
C93 = 3,4-Dihydroxy-phenothyl-3-O- β -D-glucopyranoside,
C94 = N-cis-Caffeoyl-Tyramine, **C95** = 3'-O-Methyl-brazilin,
C96 = Isotachioside, **C97** = 4'-O-Methyl-Brazilin, **C98** = Moracin G,
C99 = Norbergenin, **C100** = Smilaxin,
C101 = 1,5-Bis-(4-hydroxy-3-Methoxy-phenyl)-1,4-pentadien-3-one,
C102 = Meliadoside B, **C103** = 1-Galloyl- β -D-glucose,
C104 = Osmanthuside H, **C105** = Quercetin-3-O-xyloside,
C106 = D-glucoside, **C107** = Forsythoside D,
C108 = 6-Di-O-galloyl- β -Dglucose,
C109 = 2,4,6-Trihydroxy-acetophenone-2,4-di-O- β -D-Glucopyrano
side, **C110** = 3,5-O-DicaffeoylQuinic acid, **C111** = Mulberrofuran Q,
C112 = Neosappanone A, **C113** = Tribulusamide B,
C114 = β -Hydroxy-acteoside,
C115 = Nevadensin-7-O-[α -Lrhamnosyl(1 6)]- β -D-glucoside,
C116 = Campneoside I, **C117** = 2'-Acetyl-acteoside, **C118** = p-Cresol,
C119 = Protocatechuic aldehyde, **C120** = 6-Gingerol, **C121** = Ginkgol,
C122 = Chlorogenic acid, **C123** = Methyl-5-Ocaffeoylquinic acid,
C124 = 5-O-Methylshanciguol,
C125 = 2'-Hydroxy-3',4'-dimethoxy-isoflavan-7-O- β -D-glucoside,
C126 = Kukoamine A,
C127 = (3R,4R)-3,4-trans-7,2',3'-Trihydroxy-4'-methoxy-4-[(3R)-2',
7-dihydroxy-4'-methoxyisoflavan-5'-yl]-isoflavan,
C128 = Scroside D, **C129** = Geraniin, **C130** = 2-Ethyl-4,5-dimethylphenol,
C131 = Kakuol, **C132** = 2-Octylphenol,
C133 = 2,6-Di-tert-butyl-4-hydroxy-toluene,
C134 = 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one,
C135 = Flavanthrinin, **C136** = Shogaol, **C137** = Brazilein,
C138 = 6-Gingerdione, **C139** = Dihydrocaffeoyltyramine,
C140 = Yakuchinone B, **C141** = Yakuchinone A,
C142 = 7-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-
4E,6Eheptadien-3-one, **C143** = Thanniligna, **C144** = Renifolin,
C145 = Feroxin A, **C146** = Meliadoside A, **C147** = Mulberrofuran N,
C148 = Mulberrofuran B,
C149 = 2,3,5,4'-Tetrahydroxystilbene-2-O- β -D-glucopyranoside,
C150 = Mulberrofuran D, **C151** = Cinchonain Ia, **C152** = Pseudoaspidin,

C154 = Dendrocandin B, **C154** = Isohypericin,
C155 = (3R,4R)-3,4-trans-7,2'-Dihydroxy-4'-methoxy-4-
 [(3R)-2',7-dihydroxy-4'-methoxyisoflavan-5'-yl]-isoflavan,
C156 = Forsythoside A, **C157** = Gemin D, **C158** = Furosin,
C159 = (-)-Suspensaside B, **C160** = Pedunculagin_1, **C161** = Cistanoside A,
C162 = Procyanidin C-1, **C163** = Pyrogallic acid, **C164** = Feroxidin,
C165 = Xanthoxylin, **C166** = Dengibsin, **C167** = Dendroflorin,
C168 = Caesalpins P, **C169** = Moracin C, **C170** = Demethoxycurcumin,
C171 = Feralolide, **C172** = Eckol,
C173 = 2,7-Dihydroxy-4-methoxyphenanthrene-2-O-glucoside,
C174 = Moracin M-3'-O- β -Dglucopyranoside,
C175 = Dihydrophenanthrene, **C176** = Kuzubutenolide A, **C177** = DO 20,
C178 = Phlorofucofuroeckol A,
C179 = 1,2,6-Tri-O-galloyl- β -Dglucopyranoside;
C180 = Agrimol C, **C181** = Tubuloside D, **C182** = tran-Ferulaldehyde,
C183 = Taramine, **C184** = Xanthohumol,
C185 = Torachryson-8-O- β -Dglucopyranoside,
C186 = 3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside,
C187 = Digupigan A, **C188** = 2,7-Dihydroxy-1-(4'-hydroxybenzyl)-4-
 methoxy-9,10-dihydrophenanthrene-4'-O-glucoside,
C189 = 1-O-Methyl-3,5-Odicaffeoylquinic Acid methyl ester,
C190 = Erigoster A, **C191** = Mulberrofuran P, **C192** = Mulberrofuran M,
C193 = Blestrianol B, **C194** = Tubuloside E, **C195** = Tubuloside C,
C196 = N-Methyltyramine, **C197** = Isomucronustyrene,
C198 = Magnaldehyde B, **C99** = Isoscoparone, **C200** = Protosappanin C,
C201 = Moupinamide, **C202** = Erianin, **C203** = Nobilin B,
C204 = Octahydrocurcumin, **C205** = Benzopyran derivative II,
C206 = Blestrianol C, **C207** = 3''-O Methylrenatoside,
C208 = Echinacoside, **C209** = Cistanoside B, **C210** = Formononetin,
C211 = Rubrofusarin, **C212** = Karenin, **C213** = Isoetin,
C214 = 3-Methoxy-distylin, **C215** = Smiglanin,
C216 = 5,7-Dihydroxy-6-methoxyl-8-methyl-3-(2',4'-dihydroxy-benzyl)
 -chroman-4-one, **C217** = 5-Hydroxy-7,8-dimethoxy-6-methyl-3-(3',4'-
 dihydroxy-benzyl)-chroman-4-one, **C218** = Noranhoyicaritin,
C219 = Licoricone, **C220** = Gardenin E, **C221** = Cassiaside,
C222 = Kuwanon E, **C223** = Ononin, **C224** = 3,5,6,7,8,3',4'-Heptemethoxy-
 Flavone, **C225** = (-)-Epiatzelechin-3-O- β -D-Allopyranoside,
C226 = Apigenin-7-O- β -D-glucuronopyranoside,
C227 = (2S)-5,7-Dihydroxy-6-methoxy-flavanone-7-O- β -
 D glucopyranoside, **C228** = Cyanidin-3-glucoside,
C229 = Catechin 7-O- β -Dglucopyranoside,
C230 = Delphinidin-3-glucoside, **C231** = Prim-O-Glucosyleimifugin,

C232 = 6-Methoxykaempferol-3-O- β -Dgalactopyranoside,
C233 = Silydianin, **C234** = Quercetin-3-Oglucuronide6"-methylester,
C235 = Peonidin 3-glucoside, **C236** = Baohuoside II,
C237 = Baohuoside I, **C238** = Pinocembrin-7-Neohesperidoside,
C239 = 3',4',7-Tribenzylsappanol, **C240** = Viscumneoside I,
C241 = Delphinidin-3,5-diglucoside, **C242** = Kuwanon L,
C243 = Chebuloside II, **C244** = Kuwanon K, **C245** = Cyclolaudenol,
C246 = Gypenoside XVII, **C247** = Quercetin-3-O-(6-Oferuloyl- β -D-glucopyranosyl)-(1 2)- β -D-galactopyranosyl-(1 2)- β -D-glucopyranoside, **C248** = 5-O-Methylvisamminol, **C249** = Robinetin,
C250 = Capillarisin, **C251** = Rhamnetin, **C252** = Ophiopogonone A,
C253 = 6-Aldehyde-7-methoxy-Isoophiopogonone A,
C254 = Prim-O-Glucosyl-Cimifugin, **C255** = Pinnatifinoside I,
C256 = 5-Hydroxy-6,4'-dimethoxy-flavone-7-O- β -D-glucopyranoside,
C257 = Quercetin-3-O- α -Dglucuronide, **C258** = 6-Methoxykaempferol-3-O- β -Dgalacto-Pyranoside, **C259** = Aloeresin A,
C260 = Cryptomerin B, **C261** = DO 18,
C262 = 6"-O-p-Hydroxy-Benzoyliridin, **C263** = Sagittatoside B,
C264 = 3,4-DihydroxyPhen-Ethylamine, **C265** = Anthranol,
C266 = 2-Hydroxyphenyl-Propanol, **C267** = Eugenol,
C268 = (+)-threo-Guaiacyl Glycerol, **C269** = Homoarbutin,
C270 = Salidroside, **C271** = Dendrocandin E,
C272 = Cistanoside H, **C273** = Isocimicifugamide,
C274 = Filixic acid ABA, **C275** = Blestritin A, **C276** = Kuwanon P,
C277 = Ciwujiatone, **C278** = Asebotin, **C279** = 2-((3R,4R)-7-Hydroxy-4-(4-hydroxy-5-((R)-7-hydroxychroman-3-yl)-2-methoxyphenyl)-chroman-3-yl)-5-methoxycyclohexa-2,5-diene-1,4-dione, **C280** = Mulberrofuran C,
C281 = 2,4,6-Tri-O-galloyl- β -Dglucose, **C282** = Dryocrassin,
C283 = 1,2,4,6-Tetra-O-galloyl- β -D-glucopyranoside.

3. Results and discussion

The output of the MCA performed in this study is presented in Tables 1, 2, and Figures 1, 2, and 3.

From the 'proportion of inertia' in Table 1, the first four dimensions (Dimensions 1, 2, 3, 4) accounted for 53.7 % of the cumulative inertia, suggesting that the remaining seven dimensions accounted for 46.3 % of the cumulative inertia. Hence, these four dimensions were considered for the analysis. A further increase in the dimension had a little influence on the cumulative inertia. Sta-

tistically, it is not feasible to consider 4 dimensions in a correspondence analysis (see Camiz and Gomes (2016)) for visualization, hence, two dimensions were considered for visualization in this study. Another noticeable result to consider in Table 1 is the observed correlation between the studied variables (solvent and compounds). A correlation value of 0.322 was estimated between the variables, and such level of correlation is considered acceptable in the biological sciences even though a higher value of at least 0.50 is considered acceptable in statistics (see Belton and Stewart (2002)). This correlation value (0.322) indicates a positive relationship between the polarity of solvent used during the extraction process and the type of compounds extracted. Table 2 provided more details on the pattern of the relationship between the variables. From Table 2, a close relationship was observed between water and methanol extracts of Enoki caps (inertia = 0.576 and 0.555, respectively) while acetone and ethyl acetate extract of Enoki correlated (inertia = 0.348 and 0.323, respectively). This is an indication of the fact that solvent polarity differentiated the type of compounds extracted from the same mushroom using different solvents. Water is a highly polar solvent and methanol is a relatively less polar solvent, hence, they showed a closer relationship (inertia = 0.576 and 0.555, respectively). However, acetone and ethyl acetate are non-polar solvents, hence, the compounds extracted with acetone and ethyl acetate were similar and such, they clustered.

Similarly, the proportion of inertia in Table 2 also showed the impact of solvent polarity on the extracted compounds. A closer 'contribution of point to inertia of dimension' indicates a closer relationship between the pairs, while a wider 'contribution of point to inertia of dimension' indicates no relationship between the pairs. Considering dimension 2 under the contribution of point to inertia of dimension, water and methanol extracts of Enoki caps had a closer inertia contribution (19.5 % and 20.8%, respectively), while acetone and ethyl acetate extracts of Enoki caps related (6.7 % and 5.2% respectively). This trend was observed for the other solvent systems except for water extract of Bunapi shimeji caps which showed a distanced relationship with the other solvent systems as manifested in its higher inertia contribution of 36.9 % in dimension 2. This distanced relationship between water extract of Bunapi shimeji caps and the other solvents could be due to the peculiarities of the phytochemicals (in terms of polarity and type) contained in the mushroom compared to those contained in the other mushrooms.

Figures 1, 2, and 3 showed the graphical representation of the relationship between the solvents and the extracted compounds from the mushrooms. From Figure 1, five different clusters can be observed (circled). Cluster 1 is formed by the phytochemicals extracted from Enoki caps using water and methanol while, while Cluster 2 is formed by the phytochemicals extracted from Enoki

caps using acetone and ethyl acetate. The observed distance between the clusters showed that both clusters contained different groups of phytochemicals extracted with different solvents of varying polarities. Cluster 3 is formed by the clustering of phytochemicals extracted from Buna shimeji caps (using water, methanol, acetone, and ethyl acetate) and Bunapi shimeji caps (using acetone and ethyl acetate). This clustering showed that the type of solvent used during the extraction process was not showing enough differentiation of the extracted compounds from the mushrooms. Water (Cluster 4) and methanol (Cluster 5) extracts of Bunapi shimeji caps were separated from the other clusters likely due to the extraction of certain compounds from Bunapi shimeji caps using water and methanol as solvents. Possibly, such compounds were lacking in the acetone and ethyl acetate extracts of Bunapi shimeji caps, hence, they were separated from Clusters 4 and 5.

A patterned clustering of the fractions could also be observed in Cluster 3; water and methanol extracts of Buna shimeji caps clustered in the lower plane (red arrow in Figure 1) while acetone and ethyl acetate extracts of Buna shimeji caps clustered in the upper plane (red arrow in Figure 1). This pattern of clustering evidenced a positive correlation between the pairs. Statistically, pairs that are separated from each other are negatively correlated and this could explain the separation of acetone and ethyl acetate extracts of Bunapi shimeji caps from the rest of Cluster 3.

Figure 2 showed the clustering of the compounds extracted with water, methanol, acetone, and ethyl acetate from Enoki caps, Buna shimeji caps, and Bunapi shimeji caps (\mathbf{C} = compounds while 1 to 283 represents the compounds). The names of the compounds represented by \mathbf{C} in Figure 2 are provided as a supplementary file. In Figure 2, the compounds were noted to cluster in a similar trend as observed in Figure 1. A heavy clustering was observed in the right upper (depicting Cluster 2) and lower quadrants (depicting Cluster 3 in Figure 1), while less clustering was observed in the left upper quadrant (depicting Cluster 1) and left lower quadrant (depicting Clusters 4 and 5) in Figure 1. The placement of the clusters in different quadrants portrays the type of relationship that exists between the components of such clusters. Clusters in the right upper quadrant are negatively correlated with those in the left quadrant, while clusters in the same quadrant are positively correlated (see Lever *et al.* (2017)). Figure 3 depicts a combination of Figures 1 and 2. The figure presented a 2D view of the correlation between the solvents used during the extraction process and the extracted compounds. The figure also showed the 5 clusters identified in Figures 1 and 2, where compounds from Clusters 1, 4, and 5 clustered in the left upper and lower quadrants, while compounds from Clusters 2 and 3 clustered in the right upper and lower quadrants.

With these clustering patterns, it is thought that solvent polarity played a role in determining the type of compound extracted from each mushroom. The polar solvents (water) and semi-polar solvent (methanol) extracted both polar and non-polar phytochemicals from the mushrooms compared to the non-polar solvents (acetone and ethyl acetate) which extracted only non-polar phytochemicals from the mushrooms. Hence, the number of phytochemicals extracted from the mushrooms using water and methanol was more than the number extracted using acetone and ethyl acetate. For instance, water extract of Enoki caps contained 51 phytochemicals among which are C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, while methanol extract of Enoki caps contained 51 phytochemicals, including C52, C53, C54, C55, C56, C57, C58, C59, C60 and C61. Acetone and methanol extracts of Enoki caps contained 39 phytochemicals C91, C92, C93, C94 and C95 and 35 phytochemicals, including C118, C119 and C120, respectively (refer to the Appendix section for the names of the phytochemicals). Furthermore, water and methanol extracts of Buna shimeji caps contained 42 and 48 phytochemicals, respectively which were higher than 35 phytochemicals each contained in acetone and ethyl acetate extracts of Buna shimeji. Finally, water and methanol extracts of Bunapi shimeji caps contained 50 and 48 compounds, respectively, which were higher than 37 and 42 phytochemicals contained in acetone and ethyl acetate extracts of Bunapi shimeji caps, respectively. The names of all the phytochemicals extracted from all the mushrooms have been provided in subsection 2.2 while the complete list of phytochemicals extracted from each mushroom has been provided in Table 3. Generally, water and methanol extracts of the mushrooms contained more of polar phytochemicals such as C2, C3, C8, C15, C16, C27, C55, etc compared to acetone and ethyl acetate extracts. The variations in the number of polar phytochemical contents of the extracts gave rise to the clustering pattern observed in Figure 3.

These observations agreed with the previous reports of Shah *et al.* (2018) and Ukaegbu *et al.* (2018, 2019), where water and methanol were presented as the ideal solvents for the extraction of phytochemicals from edible mushrooms compared to the non-polar solvents. As per Visht & Chaturvedi (2012), mushroom phytochemicals can be easily extracted using polar solvents such as water and methanol due to the presence of a dipole force interaction between the solvent and the OH- in the phytochemicals. Such an interaction facilitates the solubility of compounds in polar solvents compared to non-polar solvents.

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Table 1: Summary table of the MCA

Dimension	Singular Value	Inertia	Chi Square	Sig.	Proportion of Inertial		Confidence of SV	
					Accounted for	Cumulative	SD	Correlation
1	0.926	0.857			0.167	0.167	0.006	0.322
2	0.848	0.720			0.140	0.307	0.017	
3	0.784	0.615			0.120	0.427		
4	0.750	0.563			0.110	0.537		
5	0.727	0.529			0.103	0.640		
6	0.685	0.469			0.091	0.731		
7	0.615	0.378			0.074	0.805		
8	0.548	0.301			0.059	0.864		
9	0.522	0.272			0.053	0.917		
10	0.472	0.222			0.043	0.960		
11	0.452	0.204			0.040	1.000		
Total		5.130	2805.879	1.000a	1.000	1.000		

a = 3102 degrees of freedom.

Table 2: Overview of row points (symmetrical normalization)

Solvent	Mass	1	2	Inertia	Contribution of				Total
					Point to Inertia of Dimension		Dimension to Inertia of Point		
					1	2	1	2	
ECW	0.093	-1.015	1.333	0.576	0.104	0.195	0.155	0.244	0.398
ECM	0.093	-0.864	1.377	0.555	0.075	0.208	0.116	0.270	0.386
ECA	0.084	0.055	0.824	0.348	0.000	0.067	0.001	0.139	0.140
ECEA	0.080	0.200	0.738	0.323	0.003	0.052	0.009	0.115	0.124
BCW	0.084	1.110	-0.326	0.426	0.112	0.011	0.225	0.018	0.243
BCM	0.084	1.171	-0.389	0.335	0.125	0.015	0.319	0.032	0.351
BCA	0.077	1.054	-0.255	0.334	0.092	0.006	0.237	0.013	0.249
BCEA	0.093	0.987	-0.191	0.395	0.098	0.004	0.213	0.007	0.220
BPCW	0.090	-1.752	-1.870	0.649	0.297	0.369	0.392	0.410	0.802
BPCM	0.079	-0.998	-0.601	0.423	0.085	0.033	0.171	0.057	0.228
BPCA	0.066	0.05	-0.480	0.370	0.000	0.018	0.000	0.035	0.035
BPCEA	0.077	0.323	-0.488	0.395	0.009	0.022	0.019	0.039	0.058
Active total	1.000			5.130	1.000	1.000			

Note: ECW=water extract of Enoki caps, ECM=Methanol extract of Enoki caps, ECA=Acetone extract of Enoki caps, ECEA=Ethyl acetate extract of Enoki caps, BCW=Water extract of Buna shimeji caps, BCM = Methanol extract of Buna shimeji caps, BCA=Acetone extract of Buna shimeji caps, BCEA=Ethyl acetate extract of Buna shimeji caps, BOCW=water extract of Bunapi shimeji caps, BPCM=Methanol extract of Buna shimeji caps, BPCA = Acetone extract of Buna shimeji caps, BPCEA = Ethyl acetate extract of Buna shimeji caps.

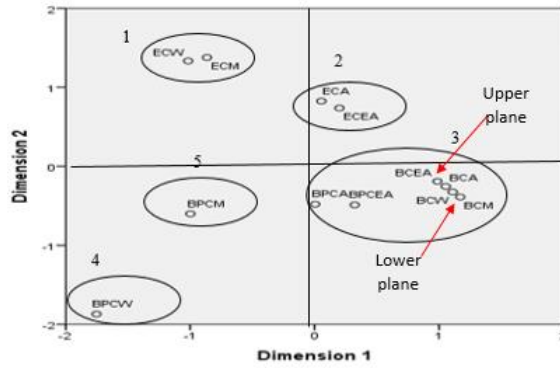


Figure 1: Row points for solvent

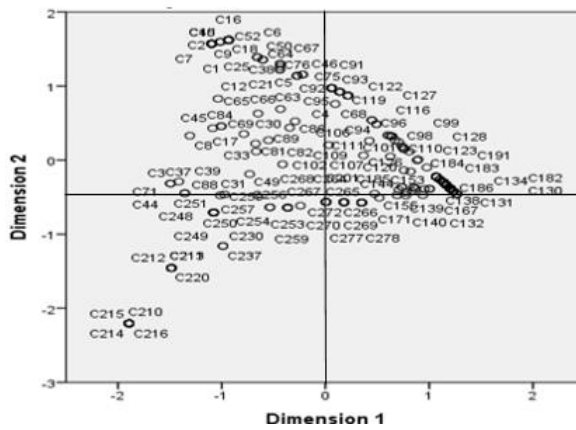


Figure 2: Column points for compounds

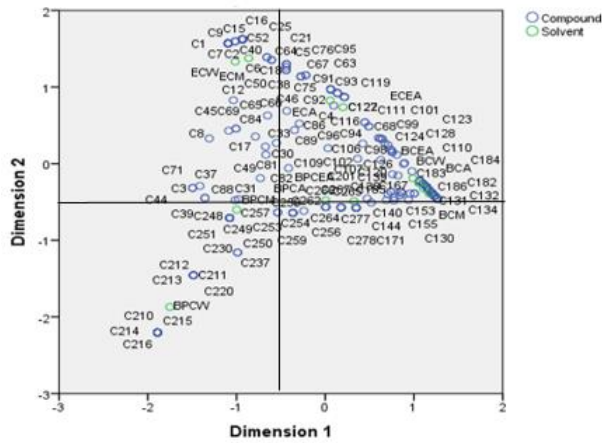


Figure 3: Row and column points for solvent and compound

Statistical Analysis of Solvent Polarity Effects on Phytochemicals Extraction

Table 3: Identified phytochemicals from mushrooms extracts prepared with different solvents

	<i>Water</i> (51 compounds)	<i>Methanol</i> (51 compounds)	<i>Acetone</i> (39 compounds)	<i>Ethyl acetate</i> (35 compounds)
EC	C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, C26, C27, C28, C29, C30, C31, C32, C33, C34, C35, C36, C37, C38, C39, C40, C41, C42, C43, C44, C45, C46, C47, C48, C49, C50, C51	C52, C53, C54, C55, C6, C56, C57, C8, C58, C59, C12, C60, C61, C15, C62, C17, C18, C21, C63, C64, C65, C66, C25, C67, C68, C69, C70, C71, C72, C73, C30, C74, C75, C33, C76, C77, C78, C40, C79, C80, C81, C82, C83, C84, C85, C86, C87, C88, C89, C90, C50	C91, C92, C93, C94, C95, C96, C4, C97, C98, C99, C100, C101, C102, C103, C17, C18, C63, C64, C104, C67, C68, C105, C106, C75, C107, C108, C76, C109, C110, C111, C112, C113, C114, C115, C116, C47, C117, C89, C50	C118, C119, C120, C94, C95, C96, C121, C99, C5, C102, C122, C123, C17, C68, C105, C124, C106, C75, C125, C33, C107, C38, C126, C127, C112, C113, C114, C115, C116, C117, C89, C128, C50, C51, C129
	<i>Water</i> (42 compounds)	<i>Methanol</i> (48 compounds)	<i>Acetone</i> (35 compounds)	<i>Ethyl acetate</i> (35 compounds)
BSC	C130, C131, C132, C133, C134, C135, C136, C137, C138, C120, C139, C96, C4, C121, C140, C141, C99, C142, C102, C143, C144, C145, C123, C146, C147, C148, C149, C150, C151, C152, C153, C154, C126, C155, C156, C157, C158, C159, C160, C161, C128, C162	C163, C130, C164, C165, C132, C166, C167, C136, C138, C120, C168, C139, C169, C141, C170, C171, C145, C123, C172, C146, C147, C173, C174, C149, C150, C151, C175, C176, C107, C153, C154, C110, C126, C177, C155, C178, C156, C157, C179, C113, C114, C158, C180, C159, C161, C128, C162, C181	C182, C165, C166, C167, C136, C183, C138, C170, C171, C184, C172, C173, C174, C185, C186, C187, C176, C188, C189, C190, C191, C192, C156, C193, C179, C158, C194, C117, C180, C159, C161, C128, C162, C181, C195	C118, C196, C197, C198, C183, C136, C19, C138, C200, C141, C201, C202, C203, C101, C170, C123, C204, C173, C174, C124, C176, C125, C107, C153, C188, C117, C189, C190, C106, C207, C208, C113, C116, C209, C195
	<i>Water</i> (50 compounds)	<i>Methanol</i> (48 compounds)	<i>Acetone</i> (37 compounds)	<i>Ethyl acetate</i> (42 compounds)
BPSC	C210, C211, C212, C3, C52, C213, C214, C8, C215, C216, C217, C218, C219, C17, C220, C221, C222, C223, C224, C225, C226, C227, C228, C71, C229, C30, C31, C230, C231, C33, C232, C233, C234, C235, C236, C37, C237, C238, C239, C240, C44, C88, C241, C242, C243, C244, C245, C49, C246, C247	C211, C248, C249, C213, C250, C251, C252, C12, C253, C220, C65, C66, C222, C69, C228, C229, C30, C31, C230, C254, C255, C33, C256, C258, C233, C234, C236, C237, C39, C259, C81, C238, C240, C241, C32, C84, C240, C44, C45, C88, C262, C263, C89, C244, C245, C49, C246, C247	C264, C265, C266, C267, C268, C138, C270, C139, C271, C102, C171, C144, C201, C185, C173, C30, C31, C230, C254, C256, C257, C107, C233, C109, C272, C273, C81, C238, C260, C274, C275, C276, C262, C89, C244, C245, C195	C265, C266, C165, C269, C138, C120, C270, C139, C140, C271, C102, C171, C144, C201, C253, C185, C277, C173, C278, C30, C106, C256, C107, C258, C188, C153, C126, C278, C259, C155, C279, C280, C274, C275, C276, C281, C262, C209, C282, C283, C49, C195

Note: EC = Enoki caps, BSC = Buna shimeji caps, BPSC = Bunapi shimeji caps.

4. Conclusion

The MCA showed a weak positive correlation $r = 0.322$ between the identified phytochemicals in the mushroom extracts and the solvents used during the extraction process possibly due to the overlapping of phytochemicals extracted from different mushrooms using different solvents. The pattern of the phytochemicals clustering suggested that solvent polarity differentiated the groups of phytochemicals extractable from the mushrooms. Water and methanol presented as the best solvents for the extraction of phytochemicals from the studied mushrooms as they extracted more phytochemicals (comprising of both phenols and flavonoids) from the mushrooms, while acetone and ethyl acetate extracted more of only flavonoids from the mushrooms. The observation could be due to the ability of water and methanol to extract both the polar and non-polar phytochemicals from the mushrooms compared to acetone and ethyl acetate which extracted more of only the non-polar phytochemicals. It is, therefore, concluded that MCA is a valid statistical tool for establishing the influence of solvent polarity on the extraction of phytochemicals from the studied mushrooms.

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