UNIVERSIDAD DE CÓRDOBA

DEPARTAMENT OF PHYSICAL CHEMISTRY
AND APPLIED THERMODYNAMICS

BIOREFINERY DESIGN BASED ON THE VALORIZATION OF FOOD INDUSTRY WASTES

BIORREFINERÍA INTEGRAL PARA LA PRODUCCIÓN DE ACEITE MICROBIANO A PARTIR DE RESIDUOS DE LA INDUSTRIA AGROALIMENTARIA

Thesis submitted to achieve the Degree of Doctor in Advanced Computing, Energy and Plasmas.

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TITULO: *BIOREFINERY DESIGN BASED ON THE VALORIZATION OF FOOD INDUSTRY WASTES*

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TÍTULO DE LA TESIS: Biorefinery design based on the valorization of food industry wastes

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INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

El doctorando ha mostrado una capacidad investigadora excepcional, que se ha plasmado en un número significativo de publicaciones en revistas de alto impacto internacional (muchas aún pendientes de publicar), así como en ponencias en congresos de investigación de reconocido prestigio, nacional e internacionalmente (según se adjunta en los indicios de calidad y en el CV del doctorando). Así, en el prestigioso congreso WasteEng2016, celebrado en Albi, Francia, obtuvo el reconocimiento "Poster Award Breakthrough Initiative". Entre sus publicaciones más relevantes se encuentran las siguientes (todas ellas relacionadas con su tesis):

-Carmona-Cabello M, Sáez-Bastante J, Pinzi S, Dorado MP. Optimization of solid food waste oil biodiesel by ultrasound-assisted transesterification. Fuel 115817. 2019
-Chebbi H, Leiva-Candia D, Carmona M, Jahouani A, Dorado MP. Biodiesel production from microbial oil provided by oleaginous yeasts from olive oil mill wastewater growing on industrial grylcerol. Industrial Crops and Products (artículo invitado) 139: 111535. 2019
-Carmona-Cabello M, Garcia IL, Leiva-Candia D, Dorado MP. Valorization of food waste based on its composition through a biorefinery. Current opinion in green chemistry 14: 67-79. 2018
-Carmona-Cabello M, Leiva-Candia D, Castro-Cantarero JL, Pinzi S, Dorado MP. Valorization of food waste from restaurants by transesterification of the lipid fraction. Fuel 215: 492-498. 2018
-Carmona-Cabello M, Garcia IL, Saez-Bastante J, Pinzi S, Koutinas A, Dorado MP. Food waste from hospitality sector - characterization for biorefinery approach. Bioresource Technology (en prensa).
-Saez-Bastante J, Carmona-Cabello M, Villarreal-Ornelas E, Trejo-Calzada R, Pinzi S, Dorado MP. Feasibility of the use of chicalote assisted with clean energies as new bioenergy for diesel engines. Industrial Crops and Products (en prensa)

Los logros alcanzados con esta tesis contribuirán notablemente al avance de la ciencia en uno de los aspectos clave del desarrollo de las biorrefinerías, al concluir con una propuesta de valorización de residuos agroalimentarios en base a su composición, dentro del concepto de economía circular. En este sentido, ha propuesto una herramienta, en el marco de la bioeconomía, que permite el reciclado de residuos en productos de alto valor añadido, de forma eficiente e innovadora. También ha analizado las propiedades de los combustibles obtenidos a partir de residuos y la respuesta de los motores, incluyendo las emisiones contaminantes, al usarse para alimentarlos. Trabaja muy bien en equipo y aprende técnicas complejas con mucha rapidez e interés. Su esfuerzo por realizar una investigación de calidad le ha llevado a realizar una estancia de investigación en el extranjero, en la Agricultural University of Athens, Grecia, que sin duda ha contribuido de forma notoria a complementar su formación en la valorización de residuos y el concepto de biorrefinería, lo cual se ha plasmado en un enriquecimiento notable de su trabajo investigador y ha permitido que el grupo de investigación continúe con una fructífera colaboración con el grupo de Grecia. Consideramos que el doctorando ha cubierto con creces los objetivos de su formación y que está capacitado para continuar avanzando en esta línea, dirigiendo su propia investigación y colaborando con otros investigadores en materias afines. Ha sido un alumno excelente en todos los aspectos, al igual que el trabajo que ha desarrollado. Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 18____ de __noviembre____ de _2019__

Firma del/de los director/es

Fdo.:_Mª del Pilar Dorado Pérez__ Fdo.: _Isabel López García__

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Abstract

The current environmental predicament requires the search for renewable and sustainable alternatives to fossil fuels, mainly in the transport sector. Biodiesel may replace diesel fuel using an environmentally friendly and economically efficient process, as long as the raw material employed is low-cost and derived from sustainable sources. Such an alternative feedstock from non-vegetable sources, does not compete with food market or land use sources. Annually, around 1.3 billion tons of food are discarded or wasted. This huge quantity has led researchers to search for new applications for this carbon source. Waste management is one of the most significant challenges of EU policies for the coming decades. Our changing food habits carry an inherent increase in waste nutrients, that are definitely worth recycling. The traditional method of waste management is not adapted to meet future energy and environmental requirements. A food waste-based biorefinery allows new approaches in waste recovery, while improving industrial processes.

This PhD thesis is based on a biorefinery concept and takes advantage of each nutrient that composes food waste. Food waste (FW) composition from local catering services was analyzed. The analysis showed a high moisture content, the main components were lipids (25.7-33.2, w/w), starch (16.2-29.4%, w/w) and proteins (18.3-23.5%, w/w) on a dry basis. Also, a metal profile with Na and Mg as main components was found, followed by trace elements, e.g. Zn or Fe. The variability of samples was studied using various statistical tests, and its relationship with FW typology was elucidated. The combination of both, chemical characterization and statistical study, is revealed as a good decision-making tool for further FW processing and valorization.

According to initial characterization analysis, oil included in solid food waste (SFW) was found to be feasible for use to produce biodiesel that meets European biodiesel standard EN 14214. For this purpose, Soxhlet extraction of the lipid fraction of SFW from different restaurants was carried out. Fatty acid composition was analyzed and potential differences concerning the source of SFW were evaluated through principal component analysis. Results showed that fatty acid composition of oils from SFW differs depending on the restaurant, but the range of fatty acid methyl esters (FAME) is similar to that found in vegetable oils, showing a high content of oleic acid (C18:1) and linolenic acid (C18:2). Due to high free fatty acid content, acid esterification pre-treatment was conducted, followed by a basic transesterification optimization, by both conventional and ultrasound (US) assisted reactions. Response surface methodology was selected to perform the experimental design; optimal conditions for conventional transesterification resulted in 93.23% w/w fatty acid methyl esters (FAME) conversion. Several chemical and physical properties of SFW oil biodiesel were analyzed. It was found that biodiesel fulfils the European standard EN 14214, with the exception of FAME yield, oxidation stability and glyceride content. It may be concluded, from this field trial, that oil from SFW from different restaurants may be mixed together and used to produce biodiesel.

Finally, oleaginous yeasts were evaluated via hospitality food waste fermentation. In light of the starch and protein content of food waste, controlled enzymatic hydrolysis was considered feasible, and required enzymes may be provided through solid state fermentation (SSF) using *A. awamori* and potato peel as substrate. Crude hydrolysates were subsequently used in shake flask fermentations, with oleaginous yeast *Rhodosporidium toruloides* Y-27012, attaining a total dry weight (TDW) of 32.9 g/L and a lipid content within the cells of 36.4 %. Results of *R. toruloides* fermentations in a fed-bath bioreactor showed a TDW of 53.9 g/L and a lipid production of 26.7g/L. Moreover, fatty acid profile of microbial oil from bioreactor samples revealed the presence of palmitoleic acid (C16:0) and oleic acid (C18:1) as main components. The principal component analysis (PCA) performed showed a fatty acid profile similar to soybean or solid food waste oil (SFWO). Finally, recovered microbial oil was transesterified to biodiesel, achieving a yield of 94%, which almost fulfills the European standard normative EN 14212.

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Symbols, acronyms and abbreviations

AOAC	Offcial Analyrical Chemists
AV	Acid value
a_w	Water activity
BC	Babassu cake
BOD	Biochemical oxigen demand
CFPP	Cold filter plugging point
CN	Cetane number
COD	Chemical oxygen demand
CP	Cold plasma
CSR	Corporate Social Responsibility
CV	Coefficient of variation
db	Dry basis
DW	Dry weight
e.g.	For example
EU	European Union
FAO	Food and Agricultural Organization
FAS	fatty acid synthase
FDR	Fine dining restaurant
FID	Flame ionization ditector
FP	Flash point
FW	food waste
FWH	Food waste hydrolysate
GC	Gas cromatography
GHG	Green-house gas emissions
GR	Grill restaurant
ICP-MS	Inductively coupled plasma mass spectrometry
IEA	International Energy Agency
IR	Italian restaurant
K-S	Kolmogorov-Smirnow test
LC	Length of chain
LCV	Lower calorific value
MCFA	Medium chain fatty acids
MO	Microbial oil
MSW	Municipal solid waste
OF	Organic fraction
OPEC	Organization of petroleum Exporting Countries
OXS	Oxidation stability
PCA	Principal component analysis
PP	Patato peel

RS	Reducing sugar
SFM	Sunflower meal
SFR	*Sophora flavescens* roots
SFW	Solid food waste
SFWO	Solid food waste oil
SmF	Submerged fermentation
SSF	Solid state fermentation
SUR	Solid urban residue
TKN	Total Kjeldahl nitrogen
TUD	Total unsaturation degree
TWD	Total dry weight
UCC	University campus cafeteria
UNDESA	The United Nations Departament of Economic and Social Affairs
US	Ultrasound
VO	Volatile solid
WB	Wheat straw;
WB	Wheat bran
WCO	Waste cooking oil
WMB	Wheat milling by-product

Chemical compounds

ADF	Acid detent fiber
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BHT	Butylated hydroxytoluene
C10:0	Capric acid
C12: 0	Lauric acid
C14: 0	Myristic acid
C16: 0	Palmitic acid
C16: 1	Palmitoleic acid
C18: 0	Stearic acid
C18: 1	Oleic acid
C18: 2	Linoleic acid
C18: 3	Linolenic acid
C8:0	Caprylic acid
CYS	cisteine
FA	Fumaric aid
FAME	Fatty acid methyl esters
FAN	Free amino nitrogen
Glu	Glucose
Gly	Glycerol
HMG-CoA	-Hydroxy β-methylglutaryl-CoA

IN	Inorganic nitrogen
IP	inorganic phosphorus
LA	Lactic acid
NDF	Neutral detergent fiber
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvaleric acid
SA	Succinic acid
TCA	tricloroacetic
TN	total nitrogen
VFA	Volatile fatty acids

Symbol

ϑ	Water content
ν	Kinematic viscosity
ρ	Density

CHAPTER 1.

PHD APPROACH

1.1. Scientific and technological content

Food waste generation results in an ethical and environmental problem. According to FAO, more than one third of the food produced globally is discarded or wasted [1]. FW is generated in a complicated process that comprises production chain and system, consumer participation, besides dietary habits [2]. FW may be defined as edible and non-edible food that are discarded at any step along the food chain: primary sector, manufacturing sector, logistics (food damaged during transportation), storage (expiry dates, loss of quality) and post-consumption [3, 4]. In the EU, households are estimated to produce the largest amount of FW, that is, 47 million tons, which represents 53% of total FW generation (figure 1-1). The cost associated has been estimated to be around 143 billion € [5].

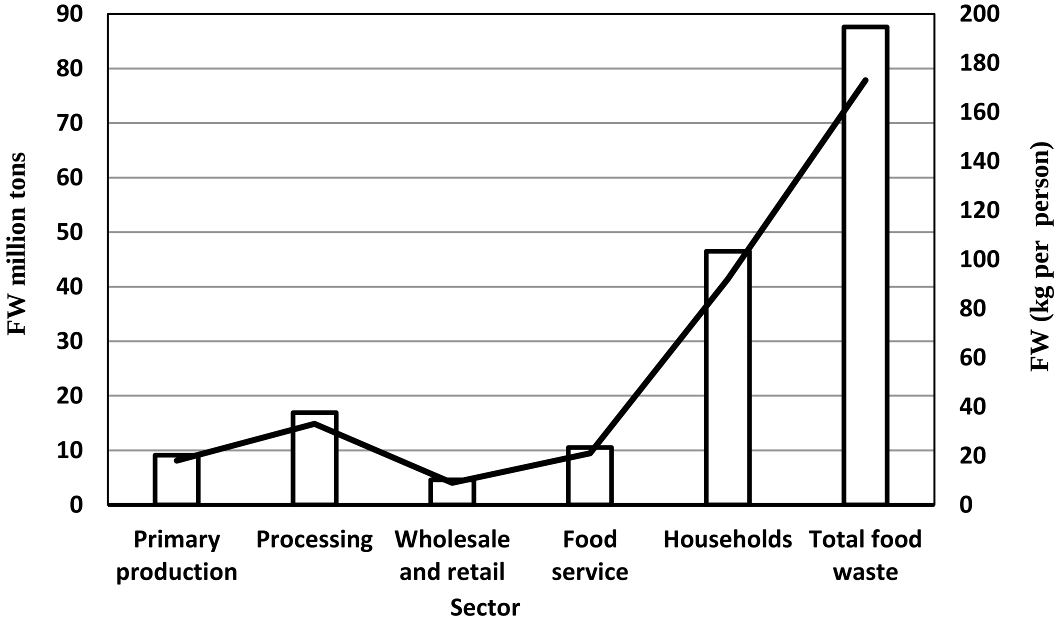

Figure 1-1. Estimates of FW generation (2012). Bars represent FW in million tons; line: FW (kg/person) in EU-28. Source: [5]

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From an environmental point of view, at a global level, FW carbon footprint represents 3.6 Gt CO_2-eq and, while in the EU, it reaches up to 1.86 Mt CO_2-eq. Factors involved in FW carbon footprint depend on food life cycle and dietary habits. As an example, vegetable food and subsequent wastes provide more greenhouse gas emissions in Europe than in industrialized Asia. However, this correlation is the inverse for cereals.

Another internal factor to take into consideration is the strong correlation between food waste quantities and carbon footprint. Exceptions of this correlation are provided by rice or meat, which exhibit a high impact due to production conditions, despite their low production volume. In this sense, rice releases methane through the decomposition of organic matter, while meat production involves methane emitted by animals, fertilizer used for production, manure management, etc. Furthermore, the carbon footprint is cumulative and depends on the food chain step. This means that greenhouse gas emissions from a spoiled tomato increases throughout the supply chain. Indeed, the carbon footprint in the consumption phase represents more than 37% of total emissions, although its contribution to total FW is below 22%. Furthermore, FW is correlated with soil acidification, contributing up to 1.66 Mt SO_2-eq, showing a high potential of eutrophication (0.74 Mt-eq) [6].

Conversely, food waste may play an economic and social role, providing new job opportunities and allowing the development of new strategies that help the viability and diversity of rural and urban economies. FW recovery can help to reduce FW environmental impact, improving food safety and recycling energy nutrients.

In this context, the biorefinery philosophy provides a similar strategy to that of petroleum-based refineries. However, biorefineries use biomass as raw-material and allow the incorporation of “corporate social responsibility” (CSR) [7-10].

A proposal of FW biorefinery is shown in figure 1-2. This is an advanced concept that aids to decarbonize the food industry, while providing an alternative to landfill (EU emissions are 99,372 kt, representing more than 90% of greenhouse gas emissions [11]) and incineration for household FW. Biorefineries may combine several technologies, allowing the production of different high value-added products. According to Chen et al. [12] over the last 20 years, there has been an exponential increase in the number of publications focused on research to develop technologies that allow FW valorization.

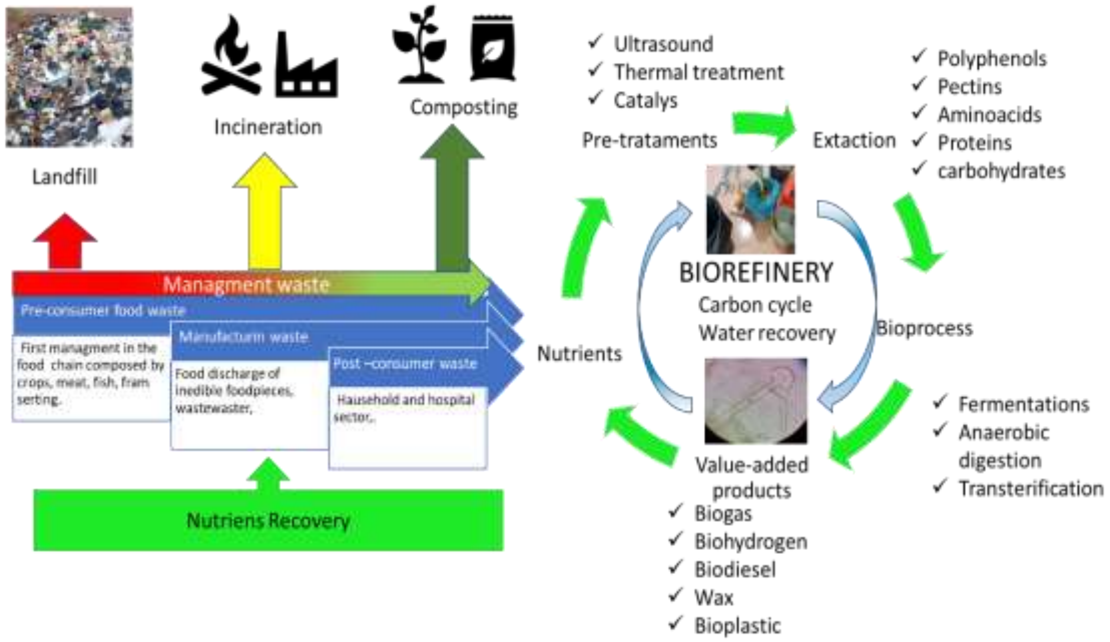

Figure 1-2. Valorization of food waste based on its composition through the concept of biorefinery. Source: [3]

With the aim of reducing the environmental impact while supplying bioenergy, biorefineries are key to build the new strategies for the next 40 years. This technology should open pathways for low-carbon energy; thus, biorefineries may become one of the main suppliers of bioenergy, together with solar and wind farms. According to the 21st session of the so-called Paris conference (United Nations Climate Change Conference, COD21), there are different future scenarios regarding the increase of global temperature, and new technologies must adapt to these scenarios. To prevent an increase of 2ºC above preindustrial levels, bioenergy should provide 17% of the final energy demand by 2060, while current values are close to 5%. Biomass for energy can be used either directly or through its conversion to biofuels and may reduce carbon dioxide by 5.7 Gt/year. According to figure 1-3, transport is expected to become the sector with the highest demand for bioenergy, with a ten-fold increase from 2015 levels, by 2060. FW-based biorefineries may develop new biofuels for aviation (bio-jet), bunker fuel for shipping or develop a new generation of advanced biodiesel or bioethanol that may be compatible with low-carbon cycle. One example of a biorefinery product is ethylene, which is used as fuel in the cement industry, and is produced from bioethanol using FW as a raw material through fermentation [13].

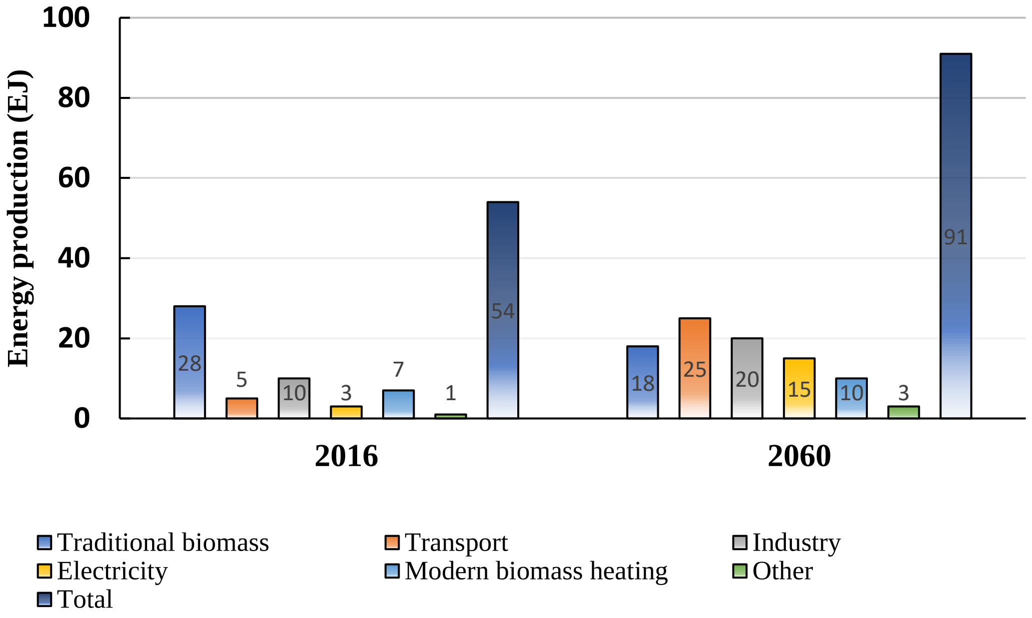

Figure 1-3 Contribution of bioenergy to final energy demand in 2015 and 2060. Source: [13]

1.2. Objectives

This doctoral thesis is focused on food waste recovery using the biorefinery concept and in a context of scientific and human interest, reflected by the large increase in studies related to food waste recovery.

Firstly, this work deals with the chemical composition and statistical variability of FW from hospitality sector. Once this composition is analyzed, a work methodology is developed with the objective of producing quality biofuel, following the principles of the bioeconomy. In sum, specific objectives are the following:

Specific objetives:

1. To study the composition of FW and analyze its role in biorefineries.
2. To characterize FW from hospitality sector and its subsequent utilization as a raw material for a biorefinery.
3. To characterize triglyceride content and fatty acid composition of solid food waste, followed by its transesterification and further biodiesel characterization, according to standard EN 14214.
4. To optimize the oil extracted from SFW by response surface modeling, using ultrasound as energy.
5. To produce biodiesel from oleaginous yeast *Rhodosporidium touruloides* Y-27012, using FW from hospitality sector as raw material.

1.3. Methodology

Planning and correct definition of methodology are the basis and starting point for addressing the contents of this thesis and, therefore, the objectives. Figure 1-4 shows schematically the structure of the methodology that was followed during the development of the work included in this document.

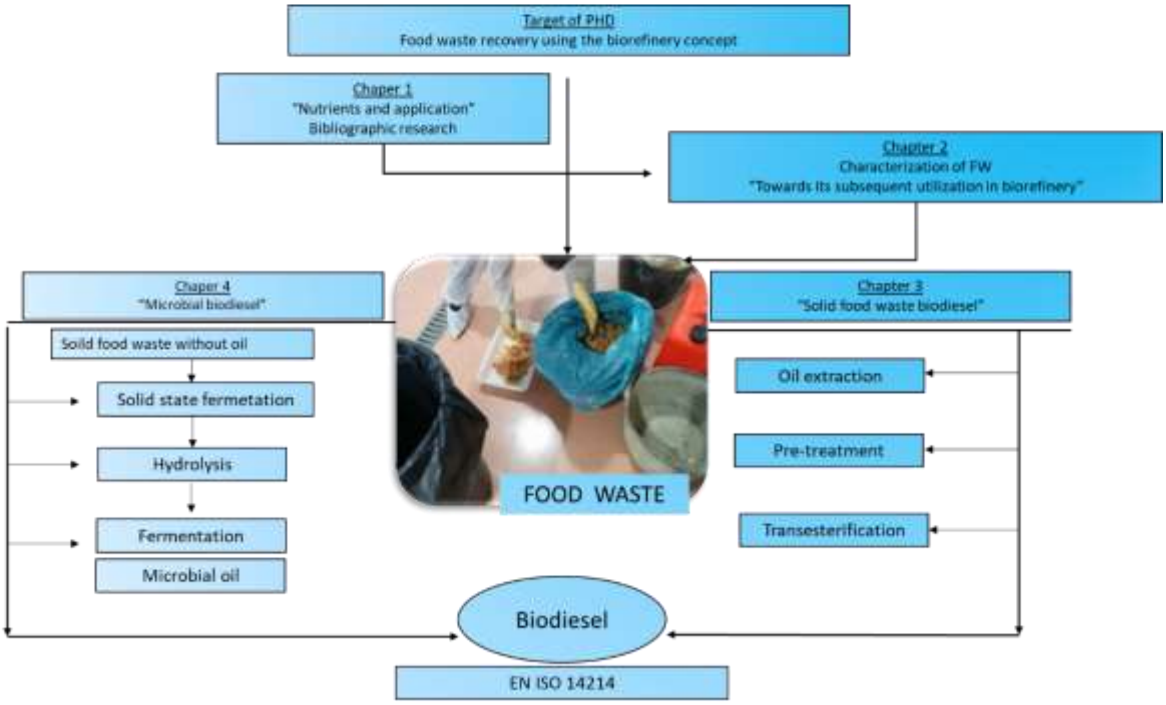

Figure 1-4. Structure of the methodology followed during the PhD development

Once the initial question which motivates the development of this thesis has been introduced, together with the objectives, the next step is to address, as in all research work, a literature review to evaluate the state of the art concerning the processes of food waste recovery. This is done in **chapter 2**, with special emphasis on food waste management from a point of view of FW nutrients, their derivates and interactions, which are of great importance for new strategies in biorefinery processes.

Since the work proposed for this thesis has a mainly empirical character, once the importance of nutrients in the recovery of waste has been established, a scale study of the hospitality sector is carried out. To assess potential biorefinery development, food waste composition derived from local catering services is studied. Moisture, lipids, starch, proteins, metal profile and trace element content are analyzed in **chapter 3.** Furthermore, a statistical variability test, in combination with a principal component analysis, allow both the establishment of specific variations in waste composition and relationship with waste typology, i.e. meat and protein variation. Combination of both chemical characterization and statistical study provides a good decision-making tool for further FW processing and valorization.

Based on results presented in **chapter 3**, two different processes are developed. Process one: extracted oil from food waste is used as raw material to produce biodiesel (**chapter 4**). Process two: other nutrients, i.e. starch, protein and fiber are used to produce microbial oil and biodiesel (**chapter 5**).

Process described in **chapter 4** is carried out in two steps; firstly, the feasibility of the use of oil, extracted from SFW from different establishments, to produce biodiesel that meets European standard EN14214 is studied. Fatty acid composition is analyzed and potential differences concerning the source are evaluated through principal component analysis. Also, oil properties, namely water content and acid value are evaluated to analyze whether an esterification pretreatment is needed. Finally, several SFW biodiesel chemical and physical properties are analyzed. Secondly, improving pre-treatment and treatment for SFW biodiesel production is studied. Due to high free fatty acid content, acid esterification pre-treatment is conducted, followed by a basic transesterification optimization. Reaction time is improved by assisting transesterification with ultrasound (US). Response surface methodology is selected to perform the experimental design.

Finally, **chapter 5** focuses on the valorization of residues from food service sector to produce biodiesel through fermentation, using oleaginous yeasts, which accumulate intracellularly lipids. In light of the starch and protein content of FW, controlled enzymatic hydrolysis is considered as a feasible alternative, and required enzymes are provided through solid state fermentation using *A. awamori* and potato peel as substrate. As result of the combination of SSF enzymes and further hydrolysis, a fermentation medium rich in carbon and nitrogen that allows microbial growth and lipid accumulation is found. Yeast *Rhodosporidium turuloides* is selected to provide microbial oil, with subsequent biodiesel production that meets European standard EN14214.

1.4. References

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CHAPTER 2.

VALORIZATION OF FOOD WASTE BASED ON ITS COMPOSITION THROUGH THE CONCEPT OF BIOREFINERY

Abstract

Waste management is one of the most significant challenges of EU policies for the coming decades. Our changing food habits carries an inherent increase in waste nutrients, that worth recycling. The traditional method of waste management is not adapted to meet future energy and environmental requirements. Food waste (FW)-based biorefinery allows new approaches in waste recovery, while improving industrial processes. However, interactions between FW nutrients, their derivates and subsequent treatment methodologies may lead to negative effects and bioprocess low yield. This Chapter has as aims to state the importance of new strategies in biorefineries, based on FW nutrients and their interactions.

2.1. Introduction

Food waste (FW) is defined as either edible or non-edible food that is discarded in any step of the food chain: primary sector manufacturing steps, logistics (food damaged during transportation), storing operations (expiration date label, quality losses) and post-consumer steps [1]. FW from primary sector (also known as pre-consumer step) is composed of food that do not reach consumer because it is either discarded or recycled. Non-edible food pieces, food that does not comply with organoleptic standards, food from process failures or batches with microbial contamination are usually discharged during manufacturing step. Last chain step, known as post-consumer step, includes residues from household and food service sector (mainly restaurants and hospitals) and are basically composed of peels, bones, cooking oil, discharged food portions, etc.

FW is generally considered non-hazardous waste, with the exception of animal waste that is controlled by European regulation (EC) No 1069/2009. Just in 2014, 2503 million t of waste [2], derived from household and economic activities, were generated in the EU-28, which means an average per inhabitant of 4.9 t/year. From that figure, *ca.* 10% corresponds to municipal solid waste (MSW), which includes around 45-55% of FW [3]. It is worth to mention that MSW control is a crucial target for EU environmental policy.

In the past 10 years, EU waste management has dramatically changed. In 2005, 63% of MSW were disposed in landfills, while ten years later, this percentage has been reduced to 24%. Moreover, in 2005, only 11% of MSW was recycled, while ten years later it increased above 25%. EU goal is to reach 60% recycling share by 2025 [2].

In terms of energy efficiency, a waste is suitable for incineration if the lower heating value is above 3344 kJ/kg [4]. Due to FW high moisture content, lower heating value is reduced. In any case, direct energy recovery through incineration provides values in a range from 3599.21 kJ/kg to 4956.77 kJ/kg, above the threshold. However, green-house gas emissions (GHG) increase [5]. An environmentally friendly recovery approach is provided by the biorefinery philosophy [6-8]. Through the refinery concept, a biorefinery fits the processing based on the raw material, with the target of maximizing both recovered nutrients and production of high value-added products. A biomass-based biorefinery may produce fuels, electricity, combined heat and power and several products (i.e. plastics), analogously to petroleum refinery, maximizing the value derived from biomass components and intermediates. Biomass-based biorefinery helps to reduce energy costs and GHG, compared to traditional energy generation plants. Biorefineries are still under research, but they are expected to play a significant role in the future of energy, material and chemical generation. Due to its properties, FW is a solid candidate as biorefinery raw material. Hence, the objective of this work is to discuss the composition of FW and analyze its role in biorefineries, finding out nutrient synergies and their applications within biorefinery processes.

2.2. Nutrient composition of food waste

Table 1 summarizes main FW nutrients, where water content plays an essential role in processing and management. Around 74% of FW nutrients are soluble in water without needing a complex refining process [9]. Water activity (a_w) in FW is an important parameter, as it is responsible for enzymatic and microorganism activities [10]. In general, a_w range from 0.5 to 0.6 is adequate for fungi and yeast growth, while higher values (0.8-0.9) are required for bacteria [11].

Draining processes, both in industry and landfills, generate large amounts of nutrient-rich waste water. As an example, tofu manufacture generates 10 L of waste water per kg of soybean and only 0.25 kg of solids [12]. Kim *et al.* [13] characterized several waste water samples, in terms of solid particles and soluble phase composition. Authors found out that 30% N was solubilized, aqueous phase showing 40% chemical oxygen demand (COD).

Carbohydrates represent the main carbon contribution in many processes of FW recovery, playing a key role in the metabolism of microorganisms for the production of hydrogen, bioethanol [14], lactase [15] or bi-oil [16], among other fermentative products. Mono- and di-saccharides, due to their high biodegradability and direct consumption in metabolic pathways [17], are the most suitable carbon-rich substrates in fermentation processes. Unfortunately, considering industrial scale, FW does not have enough concentration of these saccharides. However, polysaccharides, i.e. starch (Table 2) and fiber (*e.g.* hemicellulose, cellulose and pectin) are available in sufficient quantities to be used on a large scale. Over the last decade, several biodegradation pathways, within the biorefinery philosophy, have been developed.

Proteins provide nitrogen for bioprocesses. Hydrolysis of protein molecules produces peptides and amino acids that are essential for microorganism growth [18]. Table 2 shows properties of nutrients and synergies between them. Nowadays, the need for finding healthier nutrients, designed to prevent diseases, has opened the door to the use of pre-consumer wastes in the form of nutraceuticals, cosmetics, pharmaceuticals or leather production processes [19]. Also, due to religious precepts in some regions, such as the ban of porcine peptides in chemical hydrolysis, the extraction of collagen, chitosan, elastin or keratin from FW is an efficient current practice [3]. As an example, enzymatic hydrolysis by extracellular bacterial proteases provides acid soluble collagen yields (from fish skin) in a range of 177g/ kg -188 g/kg [20]. Moreover, collagen hydrolysis produces peptide sequence with excellent antioxidant properties. They may be used to replace synthetic antioxidants, i.e. food industry additives [21].

Lipids conform an heterogeneous group of molecules, i.e. triglycerides (glycerol bonded to fatty acid chains), phospholipids, sphingolipids, steroids, prostaglandins or terpenes [22], which may affect biochemical C/N ratio. Microorganisms can metabolize lipids by means of β-oxidation, which provides energy to cells, thus producing intermediate metabolites. According to this, during fermentation, yeast may use lipids from the initial substrate to produce *ex novo* lipid accumulation [23]. Several lipophilic components, i.e. γ-oryzanol, phytosterols (rice bran oil), diterpenes (coffee oils), limonene oils (orange peels), β-carotenes (tomato seed), pigments or phospholipids (brown seaweed oil) are highly valued in the current market [24]. As an example, pomegranate seed oils (composed of 12-24% lipids) contain punicic acid, a polyunsaturated fatty acid with many medical and pharmaceutical applications [25]. The presence of metals in FW is minor (trace element scale). However, their presence as co-factor in several enzymatic processes may play a key role in many fermentative processes [25, 26].

Table 2-1. Food waste composition by sector

Food waste (FW) composition considering food chain steps								
Step	Fruit and vegetable	Fish	Oil	Cereal	Dairy	Meat	Root and tuber	Reference
				Dry weight (%)				
Pre-consumer	48	1	2	14	6	3	26	[1]
Post-consumer	21	1	2	52	12	6	6	
Pre-consumer nutrient composition								
	Carbohydrate	Fat	Protein	Starch	Hemicellulose	Cellulose	Lignin	
				Dry weight (%)				
Potato	100	--		100				[27]
Apple waste	48.1	--	--	--	24.4	7.2	23.5	[28]
Grape	--	--	6.1	--	21.0	30.3	17.4	
Olive	31.0	--	24.0	--	26.8	36.4	26.0	
Cherry pomace	--	--		--	10.7	12.0	--	
Sugar beet pulp	--	--	10.8	--	28.1	27.4	3.1	
Black currant	--	--		--	25.3	12.0	59.3	
Pear pomace	--	--		--	25.3	34.5	59.0	
Prickly pear	33.0	--	8.6	--		27.0	2.5	
Average	56.9	--	21.0		21.4	31.3	23.2	

Table 1 (continued)

Post-consumer nutrient composition								
	Carbohydrate	Fat	Protein	Starch	Hemicellulose	Cellulose	Lignin	
	Dry weight (%)							
Food waste	10.2	--	4.05	7.06	--	41.2	--	[29]
Food waste	19.1	--	19.10	15.6	--	2.26	--	[30]
Food waste	--	--	6.62	--	21.7	--	9.2	[31]
Kitchen garbage	16.0	18.02	15.56	--	--	16.9	--	[32]
Food waste	--		0.61	--	--	--	--	[33]
Food waste	31.0	14.0	16.90	24.0	7.7	--	17.0	[34]
University cafeteria	--	--	7.43	--	--	--	--	[35]
University cafeteria	--	--	27.0	--	--	--	--	
University dining hall	--	--	135.0	--	--	--	--	
Canadian cafeteria	--	--	27.0	--	--	--	--	
Japanese cafeteria	--	--	27.0	--	--	--	--	
South Korean dining hall	--	--	12.83	--	--	14.8	--	
Food waste	--	--	1.36	--	--	21.6	--	
Coffee ground	--	--	14.85	--	--	--	33.6	[36]
Used frying oil	28.4	19.4	21.6	--	--	3.9	--	[27]
Waste edible oil	5.3	94.5	0.2	--	--	23.21	--	
Animal fat	6	35.7	54.4	--	--	--	--	
Food waste	--	17.56	21.02	31.87	--	1.6	--	[37]
Food waste	--	19.11	18.72	35.61	--	--	--	

Table 1 (continued)

Post-consumer nutrient composition

	Carbohydrate	Fat	Protein	Starch	Hemicellulose	Cellulose	Lignin	
Food waste	59.8	15.7	21.8		--	--	--	
Food waste	50.2	18.1	15.6	46.1	--	--	--	[38]
Food waste	42.3		17.8	28.3	--	2.3	--	[39]
Food waste	62.7	18.1	15.6	46.1	--	--	--	[40]
Food waste	69	6.4	4.4		--	--	--	[41]
Food waste	35.5	24.1	14.4		--	--	--	[42]
Food waste	48.3	--	17.8	42.3	--	--	--	[34, 43]
Food waste	89.9g COD/L	--	--	2.3	--	--	--	[44]
Food industry waste	--	--	--	--	--	10.03	--	[45]
Pulp and paper industry residue	--	--	--	--	--	13.78	--	[46]
Food waste	50.48	--	--	--	2.33	12.63	8.48	[47]
Average	34.5	25.5	20.7	27.9	10.5		17.0	

Wastewater composition

	pH	BOD[1]	COD[2]	NO_3^-	NH_3	SO_4^{2-}	Total P	Cl^-	
Egyptian landfill	--	10824	15629	1.4	321	596	0.37	11387	[44]
Food waste sludge	4.6	--	31900	--	--	--	--	--	[45]
Landfill	6.2	26752	70858	149	3102	1607	167	3255	[48]

[1]BOD: Biochemical oxygen demand; [2]COD: Chemical oxygen demand

2.3. Applications to biorefinery processes

Bioprocesses using FW as raw material, within the biorefinery concept, are summarized in Table 2. They have been classified in four groups, attending to the final product: bioenergy production (i.e. bioethanol, biodiesel or biohydrogen), high value-added bio-products (i.e. bio-oil, wax, bioplastics, lactic acid, tri- and di-carboxylic acids, biosurfactants or biolubricants), isolated or extracted products with important applications in pharmaceutical and food industries, and others, including enzymatic bioremediation, nanoparticles or foam.

Biorefinery processes include relatively new technologies, i.e. solid-state fermentation (SSF), where polysaccharides and proteins are split into monosaccharides and amino acids, respectively. Tsouko *et al*. [49] produced crude enzymes from *Aspergillus oryzae* SSF, achieving protease activities of 319.l3 U/g on palm kernel cake. Free amino nitrogen (FAN) production was 5.6 mg/g with a maximum yield conversion of 27% at 50ºC, in 48 h. Submerged fermentations are based on biological enzymatic hydrolysis. FW submerged fermentations with starch conversions close to 90%, and final concentrations of 143 g/L glucose, 1.8 g/L amino acids and 1.69 g/L phosphate have been reported [9].

Other techniques used in biorefinery processes, when total conversion of biomass is not achievable, namely anaerobic digestion or hydrogen production, are based on microorganisms consortia [49, 50] or different processes combination. The latter approach has been used by Demichelis *et al.* [51] combining lactic acid (LA) fermentation (yield 0.33 g LA/g FW) followed by anaerobic digestion, for the production of 0.9 Nm^3 biogas/kg_{vs} FW. Another current alternative in biorefinery processes is the coproduction of different products, like the association of volatile fatty acids (VFA) production through biogas, hydrogen or ethanol fermentations [52].

Lipid recovery is a major concern in biorefinery systems, being addressed by separation of FW in two phases: oils and fat are extracted from solid phase for the production of biodiesel, while remaining nutrients in the liquid phase are used in fermentation processes [9]. Even though the composition of the above lipid fraction makes it suitable for biodiesel production, meeting European biodiesel standard EN 14214, high concentration of free fatty acids requires a two-step biodiesel production process, including an acid pre-esterification step. Biodiesel yields above 90% have been reported [53]. However, low oxidation stability (4 h, below 6 h-minimum threshold set by the European biodiesel standard EN 14214) due to presence of polyunsaturated fatty acids [40] have been found. Due to the amphiphilic character of lipids, the use of organic solvents in combination with Sohxlet method constitutes the most frequently used extraction methodology [10]. Barik *et al.* [22] studied the effect of exposure time and FW/solvent ratio (using 2:1 methanol/chloroform) with household FW. Best lipid yield (57.8 %) was achieved after 120 min.

Finally, extraction methodologies of high value-added nutrients have been improved. New technologies, i.e. ultrasound (US) or cold plasma (CP) have replaced conventional techniques based in the use of solvents or thermal treatment [54]. The use of these techniques allows reducing environmental impact, thus helping to reduce costs. In fact, these methodologies increase process efficiency, i.e. macro and micro-molecule separation, extraction, purification, isolation. In fact, CP may be used to decontaminate and sterilize at low temperature, due to the production of ultraviolet radiation. Besides, CP may control FW odor and enzyme inactivation [54]. US may be involved in extraction processes of phenols, sugars, pigments and organic acids. Energy transmission through US supports physical processes, i.e. nanoencapsulation or fiber degradation of FW [55]. There are also sinergies between methodologies, i.e. electro-fermentation bioprocess. This is a hybrid methodology that combines conventional fermentation with microbial electrochemical system, allowing overcoming challenges at industrial scale (i.e. thermodynamic limitations). Electrodes may help reducing activation energy, thus improving catalytic effect of fermentative processes. This methodology has been able to extract higher amount of volatile fatty acids (VFA) from FW from canteen [56].

Table 2-2. Food waste-based biorefinery processes

Bio-products								
Product	**Substrate**	**Nutrient**	**Pre-treatment**	**Enzyme**	**Microorganism**	**Production yield (g/L)**	**Notes**	**Reference**
Propionic oil	Cheese whey	--	--	--	*Propionibacterium acidipropionici*	135	Inhibited by low acid level	**[57]**
Lactic acid (LA)	Wheat and rice bran	--	Enzymatic hydrolysis	--	--	129	No nutrient addition needed	**[58]**
Lactic acid	Mango peels		Hydrolyzed steam explosion		*Lactobacillus casei*	63.3		**[59]**
Lactic acid	Jackfruit	Glucose	Enzymatic hydrolysis	Glucoamilase and α-amilase	*Strectococcus equinus*	109	240 g/L initial concentration	**[60]**
Lactic acid	Food and bakery waste	100 g/L of glucose 80 g/L glucose and 7.6 g/L fructose	Solid state fermentation (SSF) by *Aspergillus awamori* and *Aspergillus oryzae*	Cellulase, protease and amylase	*Lactobacillus casei*	82.6	10 g/L yeast extract supplement. Lipid fraction removal	**[61]**
Lactic acid & Anaerobic digestion	Food waste	80 g/L glucose, 0.4 g/L FAN^3	Double process, SSF^2 and separated hydrolysis fermentation	--	*Streptococus sp.*	0.33 g LA/g FW 0.6 Nm^3 biogas/kg FW	--	**[51]**

Table 2 (continued)

Bio-products

Product	**Substrate**	**Nutrient**	**Pre-treatment**	**Enzyme**	**Microorganism**	**Production yield (g/L)**	**Notes**	**Reference**
Lactic acid	Food waste & *Sophora flavescens* roots)	--	--	--	*Lactobacillus casei*	--	pH control, 2:1 FW:SFR[1]	
Fumaric acid (FA)	--	Glucose and starch	Hydrolyzed	--	*Rhizopus oryzae*	85 g/L	--	[62]
Cellulose	FW	--	Acid hydrolysis	--	*Acetobacter xylinum*	1.09 g/L	--	[63]
Cellulose	FW	--	Enzymatic hydrolysis	--	*Acetobacter xylinum*	0.42 g/L	--	[51]
Citric acid	--	--	SSF and SmF[4]	--	*Penicillium / Aspergillus*	826,800 t	Total production in 2015	[64]
Citric acid	Waste cooking oil	Lipid fraction	--	--	*Yarrowia lipolytica*	31.7 g/L 0.4 g/g WCO[5]	6.5 g/L isocitric acid	[65]
Citric acid	Apple pomace and peanut	--	--	--	*Aspergillus ornatus* and *Alternaria alternata*	2.6 g/L	--	[65]
Succinic acid	--	26 g/L glucose and fructose	SSF by *Asperguillus niger* and *Rhizopus oryzae*	--	*Actinobacillus succinogenes*	27 g/L 1.18 g SA[6]/g sugar	--	[66]
Succinic acid	Cassava roots		Enzimatic hydrolysis	--	*Actinobacillus succinogenes*	151 g/L	Feed-bach conditions	[67]

Table 2 (continued)

Bio-products								
Product	**Substrate**	**Nutrient**	**Pre-treatment**	**Enzyme**	**Microorganism**	**Production yield (g/L)**	**Notes**	**Reference**
Fumaric acid	40 g/L dry apple pomace ultracentrifugated sludge	--	SmF	--	*Rhizopus oryzae*	25 g/L	--	[68]
Fumaric acid	Apple pomace	--	SSF	--	*Rhizopus oryzae*	52 g/L FA[7]/kg Apple pomace	--	[68]
Oxalic acid	150 g/L cashew apple juice	--	Submerged fermentation by *Aspergillus niger*	--		122 g/L	--	[69]
Bioplastics[8]	Wastewater treatment	--		--	*Serratia ureilytica*	54% dry cell weight	Dark fermentation	[70]
PHB[9] & PHV[10]	Pineapple- derived peel	--		--	*Ralsthonia eutropha*	1.6 g/L PHB, 2 g/L PHV	Dark fermentation	[71]
PHB	Date seed	--		--	*Cupriavidus necator*	11.77 g/L		[72]
Bio-energy								
Ethanol	Rice and wheat straw	Cellulose to glucose	Hydrolysis by *Aspergillus terreus*		*Kluyveromyces sp.*	23.23g/L	60 h fermentation	[59]
Ethanol	Potato waste	Starch to glucose	Hydrolysis by *Aspergillus niger*		*Saccharomyces cerevisiae*	37.93 g/L	72 h fermentation	[73]

Table 2 (continued)

Bio-energy								
Product	**Substrate**	**Nutrient**	**Pre-treatment**	**Enzyme**	**Microorganism**	**Production yield (g/L)**	**Notes**	**Reference**
Ethanol	Agriculture waste	--	--	Cellulose, β-glucosidase	*Saccharomyces cerevisiae*	11.1 g/L	Pre-treatment	[74]
Ethanol	Agriculture waste	--	--	Cellulose, β-glucosidase	*Saccharomyces cerevisiae*	2.5 g/L	Without pre-treatment	[74]
Hydrogen	Crude oil pipeline	Xylose	--	--	*Clostridium amygdalium C9*	2.5 mol/mol glucose	--	[75]
Hydrogen	Waste water sludge	Glucose and protein	Freezing, thawing and sterilization	--	*Clostridium bifermentans*	2.1 mmol-H_2/g-COD[11]	--	[76]
Hydrogen	Industrial food waste	--	--	--	*Clostridiaceae/ Ruminococcaceae* family	1.01.75±3.71 L H_2/kg FW	6 h of continuous stirring in bioreactor	[46]
Hydrogen	--	Glucose	--	--	--	1.82 mol/mol glucose	Estimated cost plan: 1,256,987 $ US	[77]
Hydrogen	Pulp and paper sludge / FW	--	--	--	--	64.48 mL/g H_2	Supplied energy: 0.2 kJ/g	[47]
Methane						432.3 mL/g CH_4	Supplied energy: 15.5 kJ/g	

Table 2 (continued)

Bio-energy								
Product	**Substrate**	**Nutrient**	**Pre-treatment**	**Enzyme**	**Microorganism**	**Production yield (g/L)**	**Notes**	**Reference**
Hydrogen	FW organics processing	--	Different strategies: Non-pretreatment;	--	--	42 mL/g VS[12]	Control	[78]
			Ultrasound (US);			97 mL/g VS	79 kJ/g VS	
			Heat shock;			70 mL/g VS	70ºC, 30 min	
			Acid pretreatment;			55 mL/g VS	pH 3, 24 h	
			Alkali pretreatment;			46 mL/g VS	pH 11.4, 24 h	
			US +heat shock;			78 mL/g VS		
			US+acid pretreatment			118 mL/g		
			US+basic pretreatment			67 mL/g VS		

Biodiesel					
Product	**Substrate**	**Lipid content (%, w/w)**	**FAME yield (%, w/w)**	**Notes**	
Biodiesel	WCO	--	96.4	Heterogeneous catalyst (S-ZrO$_2$), transesterification with methanol-to oil molar ratio 10:1, temperature 40ºC	[53]
Biodiesel	Solid food waste oil	26 -33	83.99 -88	High acid value, acid esterification. Transesterification conditions: methanol-to oil molar ratio 6:1, temperature 60ºC. Principal composition: C16: 0 16.72 %, C18:1: 49.99 % and C18:2 21.37 %	[79]

Table 2 (continued)

Biodiesel

Product	Substrate	Lipid content (%, w/w)	FAME yield (%, w/w)	Notes	
Biodiesel	SFWO[13]	--	Acid catalyst: 98.5 Basic catalyst: 97.8	High acid value, acid esterification. Transesterification conditions: methanol-to oil molar ratio 6:1, temperature 60ºC	[80]
Bio-oil	FW	0.321 g lipid/ g cell 0.208 g lipid/ g cell	--	Fungal: Fungal hydrolysis by *A. oryzae* and *A. awamori*. Autolysis. *Schizochytrium mongrovei:* 0.321 g lipid/ g cell // *Chlorella pyrenoidosa*: 0.208 // Conditions 25ºC, pH 6.5, 400 rpm, SmF-2L bioreactor type	[81]

Bioenergy: methane

Substrate	Pre-treatment	Process type	Yield (mL/g VS)	notes	
Fruit and vegetable waste		Two stages	530	Vessel type: bioreactor with 0.5 L, 29 fermentation days and 95.1% VS efficiency. Inoculum: cow manure	[81]
FW	Freeze drying	Two stages	482	8 L working volume vessel; efficiency: 90% VS	[82]
FW	SSF	Two stages	432	Bioreactor 5 L, 98 days	[83]
FW	Heat treatment 100ºC, 30 min	Two stages	--	80 % CH_4 Vessel: anaerobic sludge blanket reactor with 2.3 L working time: 60 Days	[84]
FW	Liquidized at 175ºC, for 1 h	One stage	--	63% CH_4 Vessel: up flow anaerobic sludge blanket reactor with 2 L working time: 72 days; efficiency: 93.7 %	[85]

Table 2 (continued)

Pharmaceutical & food industry products					
Product	**Substrate**	**Production yield (% w/w)**	**Treatment**	**Notes**	
Chitin/chitosan	Shell/ exoskeletons and endoskeleton	--	Extracted by chemical and microbial microwave irradiation	Functional food and nutraceuticals (prebiotic and antimicrobial properties, antioxidant gelation, dietary fibre, pharmaceutical &cosmetic uses)	[86-88]
Chitin/chitosan	*Illex argentinus* squid	90	Chemical and enzymatic	Treatment conditions: chemical with 0.82M NaOH and alkaline protease, pH around 9.50	[89]
Chitin/chitosan	Shrimp shells Peanaeus	30-35	Alkali chemical	Nutrients recovery from aquacultural waste. Anticancer activity in ovarian cancer	[90]
Gold, silver, Zinc, iron nanoparticles	Pre-consumer food waste	--	Oxidation /reduction with $AuCl_3$, $AgNO_3$, $Zn(NO_3)_2$ TiO_2	Biomedicine application such as proteasome inhibition, antibacterial activity, hemocompatibility	[91, 92]

Table 2 (continued)

Others					
Lactonic sophorolipid[12]	WCO	55.6 g/L	Ultrasound in feed-bach	Reduction of surface tension employed in emulsifiers and foaming agent. Microorganism: *Starmerella bombicola*	[93]
Total polyphenol content	Pomegranate peel		US	Optimal condition: solid to solvent (ethanol) ratio 14.44, extraction time 25 min and extraction temperature 80ºC	[94]
Protein recovery	Fishery industry waste	> 95 %	Assisted by US		[95]
Anthocyanin	Purple-fleshed potato	60 mg/100 g FW	Pulsed electric field	Energy used 3.4 kV/cm, 8.92 kJ/kg, 15ºC	[50]
Bioremediation	FW	34.4-87.6	Inmobilized enzyme	Effluent treated with aqueous solution (pH 7)	[50]

[1]*Sophora flavescens* roots: SFW; [2]Solid State Fermentation: SSF; [3]Free Amino Nitrogen: FAN; [4]Submerged fermentation: SmF; [5]Waste cooking oil: WCO; [6]Succinic acid: SA; [7]Fumaric aid: FA; [8]Poly-3(hydroxybutyrate-co-hydroxyvakerate); [9]Polyhydroxybutyrate: PHB; [10]Polyhydroxyvaleric acid: PHV; [11]Chemical oxygen demand: COD; [12]Volatile solid; [13]Solid food waste oil: SFWO; [14]Biosurfactant

2.4. Nutrient synergies and interactions

Concerning biorefinery bioprocesses, interaction between FW nutrients plays an important role in bioaccessibility, degradation and inhibition [96]. Table 2 summarizes reactions and interactions between nutrients (proteins, starch, carbohydrates or phenols). FW is rich in nutrients, showing a complex chemical matrix. Compartmentalization (cellular organelles, tissues structures or plan embryos), structures (muscular or vegetal fibers) or emulsion formation can retain nutrients, making necessary the use of pretreatments [97]. Lignin (a complex polymer composed by fibers) and other fibers (lignocellulose, cellulose and hemicellulose) restrict polysaccharide breakdown with an intricate structure. Delignification is a crucial parameter for economic viability of circular economy-based biorefineries. Therefore, an adequate pretreatment should focus on increasing the accessibility of microorganisms, degrading polymeric fibers into monomeric units. Current pretreatments involve four different strategies (physical, chemical, chemical-physical and biological) and their combination. Physical methods (milling, freezing, extrusion, microwave or pyrolysis) are focused on increasing the surface area, and hence the accessibility to microorganisms. Unfortunately, the process is costly. Moreover, inhibitors may be released during the process. Acid or alkaline chemical (or new ozonolysis) pretreatments may harm environment and are costly, although are frequently used. Biological treatments are environmentally friendly and are based on the use of fungi, as *Phanerochaete chrysosporium* or microbial consortium. Combined pretreatments improve processing time, sugar yield and underproduction of inhibitors. In this sense, enzyme hydrolysis together with superfine grinding with steam explosion is considered a top methodology, with a good balance between technical parameters and energy consumption [97-99].

FW nutrients are transformed throughout different stages, i.e. food processing, cooking or waste storage. During these processes, FW suffers several physical treatments (*e.g.* break of polymeric structures or reduction of particle size), thermal variations (*e.g.* induced Millard reaction [14]), structural changes (i.e. recrystallization in polysaccharides [100], lipids [97] or meat fibers [101]) and microorganism degradation. Nutrients and their derivates may produce negative effect in bioprocesses, like the inhibitor role of carbohydrates and their derivates, or enzyme inhibition caused by the presence of glucose [102]. Concentration of these substrates plays, also, a key role in bioprocessing. Thus, high glucose or fructose concentration is related to an increase of osmotic pressure and reduction of cellular nutrition [103], with lag phase prolongation [15]. Finally, hydrolysis processes are related to the formation of inhibitors, such as furan derivatives, phenols or organic acids. Furan and its derivatives are considered the most potent inhibitors of enzymatic activity or DNA damage [104]. Also, high lipid concentration may lead to methane yield reduction [50]. Inhibition may be related to the ratio between long-chain fatty acid formation and β-oxidation degradation during hydrolysis [105]. Neves *et al.* [106] tested different amounts of lipids, being added in pulses during co-digestion of cow mature, working in both continuous and batch reactions. Results showed that high lipid concentration operated in continuous feed processes leads to higher inhibition in anaerobic digestion than reported for batch assays, allowing less degradation of oils and organic materials. Protein content in FW biomass depicts a significant seasonal variation [107]. Some authors have incorporated the exact amount of nitrogen to bioprocesses to provide an adequate C/N ratio, avoiding microorganism inhibition due to nitrogen excess [108]. Thus, proteins degrade to ammonium ion (NH^+_4) or ammonia (NH_3) [8]. Low C/N ratios and FW nitrogen degradation (due to acidogenesis during fermentation) may lead to rapid acidification of medium, besides fermentation inhibition [109]. Odors are also related to N-derivates, such as NH_3, trimethylalanine or nitrous oxide (N_2O) [110].

Metals and other impurities in FW may affect biological processes (mostly enzymatic activity) or oxidative-reductive potential. Moreover, the presence of heavy metals, i.e. Cd, Pb or Zn, may inhibit amylase, cellulase or urease synthesis [26, 101]. Inhibition affects nutrient transport and membrane function [111]. Also, iron plays a significant role in hydrogen fermentative production, as it is essential in the oxidation of pyruvate to acetyl-CoA [112]. Influence of divalent and monovalent metals in α-amylase during ethanol fermentation by yeast has also been reported [109]. In this sense, results showed an activation increase with the presence of monovalent and divalent ions (K, Mg and Ca), while heavy metals were responsible of inactivation. Storage and management have also a significant impact on FW moisture content. As an example, the use of paper bags instead of plastic ones may reduce 25% FW weight as a consequence of water evaporation [5]. Environmental factors, i.e. weather temperature or natural evaporation, may change nutrient concentration and FW heating values [113].

Table 2-3. Nutrient properties and synergies

Properties	Chemical reaction	Interacting components	Effect	Reference
Three different amino acid groups: • Nonpolar, uncharged groups • Polar, uncharged group • Charged groups Bad solubility in organic solvents due to polar properties	• Esterification of carboxyl groups • Acylation of amino groups • Alkylation and acylation	Peptides and protein	High temperature leads to acrylamide production via Maillard reaction; via Strecker reaction, it leads to degradation reaction with carbonyl compounds	[114]
Two groups: • Fibrillar (fibrous) • Flooded or globular proteins Soluble in strongly polar solvents, Intermolecular hydrophobic interactions	• Thermal denaturation collagen to gelatine (cooked meat) • Millard reaction in the presence of reducing sugar • Enzyme-catalyzed • Redox (cysteine residues)	Sugar, i.e. glycoproteins	Degradation into ammonia nitrogen, free ammonia nitrogen (FAN) and NH_4^+. Produce an intracellular proton imbalance causing pH mismatch and enzymatic inhibition	[115, 116]
Solubility: good solubility in water of monosaccharides, insoluble in organic solvents due to hydroxyl groups	• Reduction to produce alcohols • Oxidation to aldonic, dicarboxylic and uronic acids • Maillard reaction • Strecker aldehydes • Melanoidins	Protein: dehydroalanine, pyridoxine	Monod model: enzymatic inhibition due to substrate	[8, 109, 114]

Properties	Chemical reaction	Interacting components	Effect	Reference
Formed amyloplastic. 70% amorphous structure and 30% crystalline structure Solubility at 80% (%): 2.59 (WS); 31 (PS). Swelling capacity at 80 ºC (ratio): 7.15 (WS); 62.30 (PS).	• Gelatinating, irreversible change between 50-70ºC, factor Involves temperature, water content and botanical origin	Lipid, protein, antioxidants	Amyloplastic attracts water (20-40 g/g starch) or individual components, i.e. proteins, sugars, etc.	[117]
• From carbohydrates degradation: furanones • From cysteine, monosaccharides, methionine: tiols, thioethers, di- and trisulfites • Pyrroles, pyridines • Phenols from phenolic acids and lignin • lactones	• Formed by thermal process or in combination with a fermentation process		Degradation from lipid, protein and carbohydrate	[118]
• Tannins form aggregates with protein, amino acid, metal ions reducing the nutrients adsorption. • Metals: positive and negative effect, interaction with expression genes (e.g. carbon fixation), aggregation induction and decreased enzymatic activity, redox equilibrium change, negative effect in soil structure (important in compost production) • Aconitic acid: present in sweet sorghum, inhibition in bioethanol production, dependent of pH • Thermochemical treatment can produce inhibition via Millard reaction; interaction between carbonyl group in sugar with amino group in amino acids • Nanoplastic in sewage sludge reduces methane yield to 14.4% with 0.2 g/L nanoplastic concentration • Phenolic derivates induce oxidative stress in bacterial cell, producing dysregulated metabolic activities				[109, 115, 118-126]

Conclusions

Before starting a new food waste (FW)-based biorefinery, it is important to gather information about FW composition, that will help to choose most adequate processing to provide feasible desired products. Current studies show how the synergy between biorefinery processes may improve their yield. The tendency to isolate nutrients aims to both increase their added value and reduce the inhibition impact in bioprocesses, i.e. when lipids are transformed into biodiesel, recovering the energy of lipids and reducing their impact on anaerobic digestion. New treatments based on new energy sources, such as ultrasound, improve nutrient extraction methods and fiber degradation. These methodologies show the potential of circular economy and gradual replacement of current energy sources and derivates, such as petroleum, for alternative renewable ones

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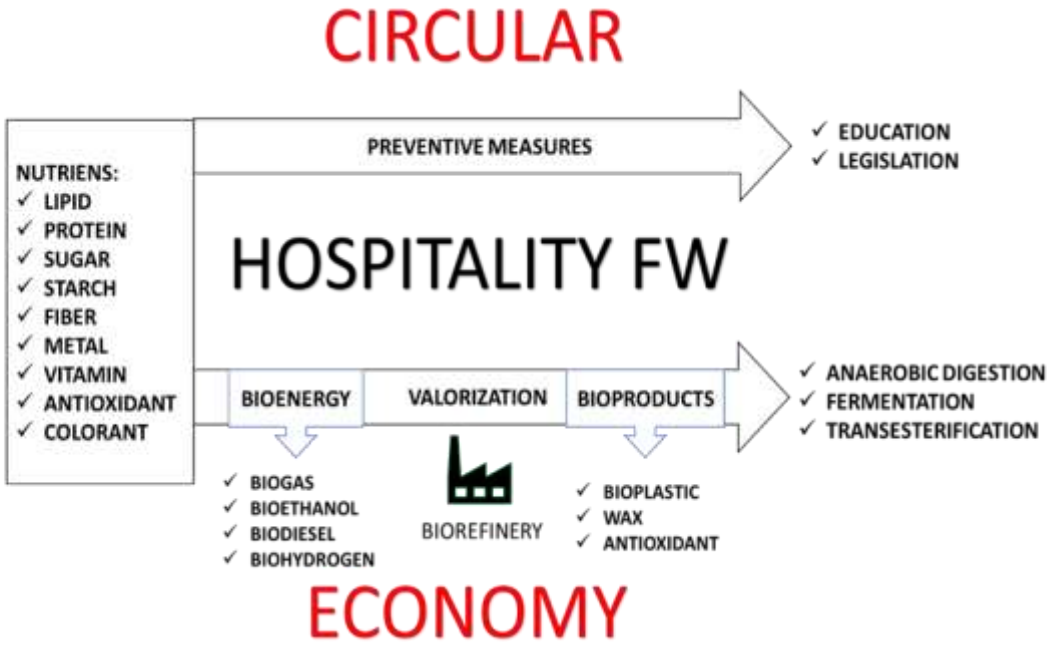
CHAPTER 3.

FOOD WASTE FROM HOSPITALITY SECTOR - CHARACTERIZATION FOR BIOREFINERY APPROACH



Abstract

High variability in food waste composition is an important problem towards the development of sustainable and robust biorefineries. The aim of this study is the analysis of food waste (FW) composition derived from local catering services to assess potential biorefinery development. The moisture content of different food waste samples showed that 27-47% (w/w) was organic material. The main components were lipids (25.7-33.2, w/w), starch (16.2-29.4%, w/w) and proteins (23.5-18.3%, w/w) on a dry basis. A metal profile with Na and Mg as main components, followed by trace elements, i.e. Zn or Fe, was also found in food waste samples. Statistical tests in combination with principal component analysis provides an efficient methodology to establish specific composition variations between FW from different catering services, while relating them to FW typology. The combination of both, chemical characterization and statistical study, shows up as a good decision-making tool for further FW processing and valorization**.**

3.1. Introduction

Among organic residues, food waste (FW) is becoming an emerging problem as a potential contaminant of water and land, as well as a source of greenhouse gas (GHG) emissions. The Food and Agricultural Organization (FAO) of the United Nations estimated that more than one third of the total produced foodstuff is lost, either discarded or not consumed [1], while other studies move this figure upwards 50% [2]. The majority of FW is generated once foodstuff reaches the distribution chain, main FW producers belong to household and hospitality sectors, the latter accounting for 21% of total generated FW around the world. Hospitality FW, originated in restaurants, hospitals, cafeterias or catering services among others, can be defined as discarded foodstuff all over the food chain: from logistics and storage processes (due to transportation damages, expired expiration date or quality losses), through cooking steps (edible, wrong processed and non-edible products, i.e. peels or bones), to not consumed food from plates or catering services leftovers. Hospitality sector FW, together with other residues, are normally collected by municipal or charter services, and accounts for 30-60% of solid urban residue (SUR) [3]. In the EU, SUR represents 7 to 10% of total generated wastes, which only in 2017 summed 246.94 million tones [4]. Around 29% SUR is recycled and 20% is treated through composting or digestion processes, though half of SUR is discharged into landfills or incinerated. However, this situation may change in the future, as European Directives 2008/98/EC (article 11) and 1999/31/EC (article 5) represent the EU commitment to reduce landfills usage to 10%, while increasing recycling routes to 65% by 2030.

Figure 1 summarizes potential recovery pathways of FW, including preventive measures and valorization processes, i.e. anaerobic digestion, fermentation and bioproducts. The utilization of FW as feedstock in industrial bioprocesses demands new governmental standards. For instance, the European Waste Framework Directive 2008/98/EC (article 6) presents the required technical specifications of FW for both composting and digestion processes. Moreover, biofuel and bioenergy production is regulated by RSB-STD-01-010 standard [5] (RSB standard for certification of biofuels based on end-of-life-products, by-products and residues), that specify levels of hygiene, impurities, odor, pH, particle size, macro/micro matter content, GHG emissions, etc. Therefore, FW-valorization in bioprocesses demands knowledge of its composition and level of degradation [6].

FW can be analyzed from different perspectives, where characterization, variability and biostability are critical issues to take into account. FW characterization includes determination of both physicochemical and biochemical properties [7]. Physicochemical property determination is focused on total organic content, e.g. C/N ratio, concentration of starch, sugar, protein, lipid, fiber, etc., as well as other analysis, e.g. pH or sample odor.

Typological analysis represents the distribution of different foodstuff groups (e.g. fruit, bread, rice, meat or meat-based food, among others) in the collected sample, allowing to express the percentage, in wet basis (w/w) of foodstuff with high fiber (i.e. peels or uneaten fruits and vegetables), starch (e.g. bread, rice, pasta) or protein (meat and fish) content [7]. Composition typology plays a key role in the study of FW variability, according to its discarding causes [8].

Variability also plays an important role in FW analysis. In fact, it is related to sample elementary composition and, consequently, the efficiency of a valorization process [8]. Main factors of variability in FW samples are related to its geographical origin, collection source (*e.g.* restaurants, cafeterias, hospitals, patisseries, etc.), FW typology and season, as well as socio-economic factors, such as income level or cultural and religious traditions [9].

Biostability defined as resistance to physical, chemical or biochemical decomposition, may be a critical factor to assess the viability of any FW valorization process. Organic matter instability generates odor and GHG emissions, as well as leachate, among other decomposing issues [10]. To date, two main biodegradation processes have been reported, namely mineralization and humidification. The former takes place when the organic fraction of matter (OF) containing lipids or carbohydrates is degraded by microorganisms, or suffers mechanical abrasion or chemical reactions (*e.g.* strecker reaction) into its elemental components, such as carbonates, ammonium, nitrate or volatile fatty acids (VFA) [11]. The second, humidification, consists on the stabilization of organic matter throughout physicochemical interactions between internal components of FW (lignin, melanin, polyphenols, etc.) and external factors, such as soil characteristics (*e.g.* porosity, soluble organic material in micropores or size of pores), in the presence of microorganisms, resulting in complex substances such as humic acid, fulvic acid, humin and its polymers [3].

The main target of the present study is the characterization of FW from hospitality sector towards its subsequent utilization as feedstock in biorefineries. For this purpose, the typological profile has been analyzed with main focus on discarding causes and chemical composition. Statistical variations have been considered. The combination of both tools could be used as a raw material selection criterion for biorefinery pathways

3.2. Materials and methods

3.2.1 Sampling

Figure 2 shows a schematic description of the sampling and characterization processes applied to FW samples.

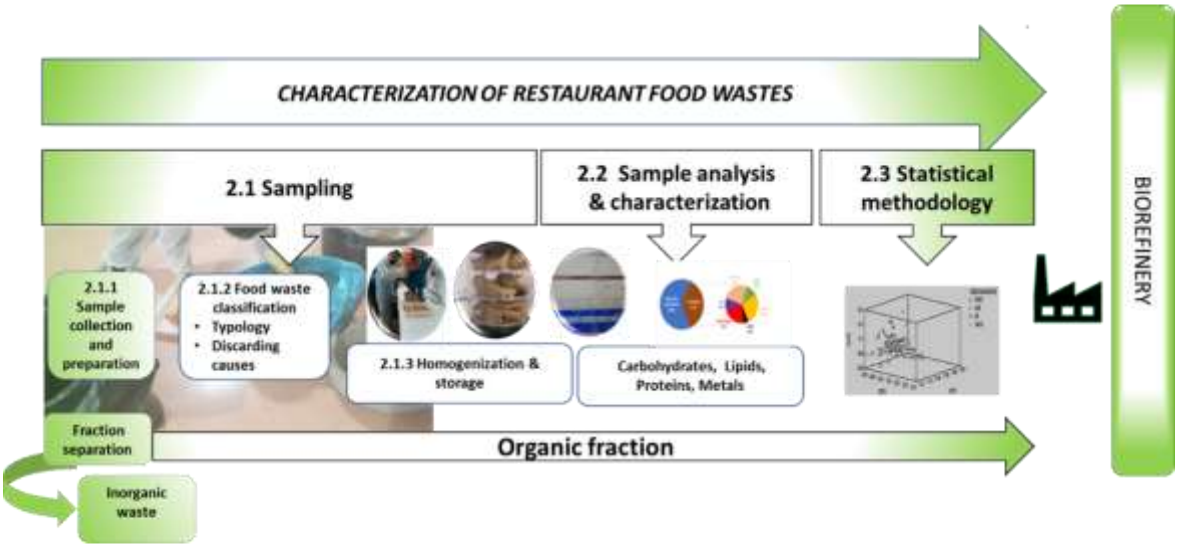


Figure 3-1. Food waste sampling treatments and characterization process.

3.2.2. Sample collection and preparation

FW samples were collected from the hospitality sector in the city of Cordoba (Spain). Four different establishments kindly supplied organic wastes from their kitchens. They were collected according to specific sampling criteria for characterization and test variability. Hospitality sector restaurants involved in the study were a grill restaurant (GR), mostly serving meat, a fine dining restaurant (FDR), offering season meals, an Italian restaurant (IR), mostly serving pasta and pizza and a University campus cafeteria (UCC), that serves seasonal daily menus and fast food. To achieve extensive sample diversity and enough representation, samples were collected in two different periods. First period encompassed a daily sampling of each restaurant during a week, accounting for 132 samples (33 samples/restaurant). The second period lasted 3 months in which samples were collected every Wednesday, accounting for 84 more samples (21 samples/restaurant); both periods resulting in a total of 216 samples. During the sample collection period, 5 months and two different stations were covered, thus analyzing the variability with respect to time

Garbage bags were picked up from restaurants according to the above described sampling plan. Inorganic residues were discarded and remaining OF was described based on the typological classification proposed by Esteves and Davlin [12].

3.2.3. Food waste classification: discarding causes

As mentioned above, FW samples were qualitatively described according to its composition (foodstuff profile). FW was therefore classified in four groups, including (1) fruit and vegetables, (2) starchy foodstuff, (3) meat, fish and byproducts and (4) others (e.g. dairy products, sweets or non-edible products, i.e. bones, shell, skin, etc.). Even though the above-mentioned authors, Esteves and Davlin [12], proposed a quantitative description based on the percentage (on a wet basis) of each foodstuff, for this study a qualitative description was adopted, followed by a further classification based on discarding causes. According to Pirani and Arafat et al.[13], four types of foodstuff discarding causes might be considered:

- Type #1: FW mainly contains out of date and non-processed food, as well as not consumed full meals. Causes of discard might be deficient logistic control, implying wrong forecasting and planning predictions about number of expected customers. Special attention to highly perishable and seasonal products (e.g. fish or special meals included in the menu) shall be paid.
- Type #2 includes surplus production of processed food, normally bounded to buffet restaurants or to a wrong forecast in hospital-, school- or prison-catering services.
- Type #3 is linked to inappropriate food handling according to hygienic, quality and storage standards, as well as legal concerns on use and handling of remaining FW. This category may contain lots of edible foodstuff, such as low-quality meats, wrong processed and discarded meals, or degraded and stinking foodstuff, among others.
- Type #4 is related to excessive meal portion sizes.

3.2.4. Homogenization

Finally, OF was ground, for a proper homogenization, using an AM80ZBA2YH mill from AEG (Germany). Samples were subsequently lyophilized for three days in a lyophilizer

LyoQuest model Telstar 55 (Spain) and stored at -20 ºC for preserver the samples of degradation by microorganism or chemical reaction.

3.3. Sample analysis and characterization

Moisture and ash content were carried out following the European Directive for Official Control of Feeding stuff [14]. Samples were dried at 105ºC and weighted several times along the process, until constant weight; moisture percentage was calculated by weight difference. For ash content calculations, samples were calcined at 550ºC and a gravimetric analysis was performed.

Total starch content was analyzed using the “Total starch” test kit from Megazyme®, usually used for the measurement and analysis of total starch in cereal flours and food products, following AOAC method no. 76.13[15], from the Association of Official Analytical Chemists (known as AOAC International). This methodology uses thermostable α-amylase (Megazyme cat. no. E-BSTAA) and amyloglucosidase (10 mL, 3,300 U/mL on soluble starch). The kit includes potassium phosphate buffer (pH 7.4), p-hydroxybenzoic acid, sodium azide (0.09% w/v), glucose oxidase plus peroxidase and 4-aminoantipyrine. Sample absorbance was measured using a Biochrom WPA lightwave II/ Visible Spectrophotometer (series number 115407, UK).

Sugars were calculated according to the “Official analysis method for juices, fruits and other vegetables and its derivates” reported in the Spanish standard RD 2081/87. The method is carried out by direct titration with sodium thiosulphate 0.1 M (0.1 N), in aqueous solution (AVS titrinorm volumetric solution, VWR BDH chemical) in the presence of phenolphthalein (99% purity, ACS reagent from Sigma-Aldrich, Germany) as indicator.

Fiber content, calculated as neutral detergent fiber (NDF), acid detent fiber (ADF) and lignin, was analyzed through the method proposed by Van Soest [16], and carried out according to AOAC official methodology no. 973.18. Fibre content may be also represented as the sum of cellulose, hemicellulose and lignin; correspondence with above mentioned calculations might be summarized as:

NDF = neutral detergent fiber

Hemicellulose = NDF- ADF

Cellulose = ADF- Lignin

Soxhlet method was used for oil quantification using a 500 mL Soxhlet apparatus. This analysis was carried out using hexane (95% purity, J.T. Baker, from Merk®, USA) as organic solvent, followed by a hexane removal step in a rotary evaporator under vacuum conditions at 65ºC. After complete removal of hexane, oil dry content was quantified by weighing difference. Liquid and solid lipidic phases were separated by a final winterization process, carried out in a temperature-controlled centrifuge (MPW-351, Poland) at 0ºC and 2000 rpm for 10 min.

Subsequently, solid and liquid oily phases were firstly quantified by weight, for each establishment and finally blended with the same phase samples from the rest of establishments. For further analysis, each phase (solid and liquid) was derived to free fatty acid methyl ester by a two-step transesterification reaction (basic and acid conditions). For the first step, sodium methylate 0.5 M (Panreac®, Spain) was used as basic catalyst, followed by HCl (37% purity, Scharlab®, Spain) for the acid step, both blended in methanol (99% purity, J.T. Baker, from Merk®, USA) with a molar ratio of 1:9.5. Both reactions took place under the following conditions: 20 min, at 70ºC and 400 rpm in a water bath and reflux column with phenolphthalein (99% purity, ACS reagent from Sigma-Aldrich, Germany) as indicator. Fatty acid profile of the transformed samples was carried out by gas chromatography using a Perking Elmer gas chromatograph Clarus 500 (Waltham, MA, USA), equipped with a Perking Elmer capillary column of 30 m x 0.25 mm Elite 5ms (0.25 µm particle diameter) and a flame ionization detector (FID).

Total Kjeldahl Nitrogen (TKN) content in solid samples was determined by AOAC method no. 955.04, using a Kjeldahl pro-Nitro M distiller from JP Selecta S.A. (Spain). Protein may be estimated as 6.25 times the obtained TKN value. In the present study, total nitrogen (TN) was reported as elemental N (by means of CHNS elemental analyzer); protein content was calculated from TKN; and the remaining nitrogen compounds were calculated as the difference of both parameters and sorted as inorganic nitrogen.

Metal content was measured by inductively coupled plasma mass spectrometry (ICP-MS) NexION 350X, from PerkinElmer (Waltham, MA, USA), while elemental analysis of C and N was performed after the Dynamic Flash Combustion Principle, using a CHNS Elemental Analyzer (Germany).

3.4. Statistical methodology

The main target of statistical analysis was the comparison between restaurant samples, to find out either similarities or differences between them, which may help defining a biorefinery based on FW valorization processes. For this purpose, IBM SPSS Statistics 22 software (USA) was used. Pearson coefficient of variation (CV) was calculated to discern the representation of the mean (dispersion). According to Fisgativa et al. [17], samples may be classified into three groups as a function of its CV: (1) samples with CV values below 16% may be included in the low variation group; (2) if CV varies from 16% to 33%, the mean would be included in the second group, considering that obtained mean shows many errors; (3) finally, if CV is above 33%, mean should not be considered as representative.

Usually, test was conducted following the protocol proposed by Ghasemi and Zahediasl [18]; lack of symmetry (skew ness) and pointiness (Kurtosis) were tested to evaluate the distribution as first criterion of normality. Furthermore, a Kolmogorov-Smirnow test (K-S) was carried out aiming to compare theoretical cumulative distribution function with obtained empirical distribution function (with a confidence level of 95%), taking into account that p-values higher than 0.05 may be considered within a normal distribution.

Also, to improve statistical description of collected samples, a non-parametric analysis was performed. Application of U-test from Mann-Whitney Wilcoxon allows to work with non-homogeneity of variance. Also, it may be used to compare means from properties that were not representative in previous tests. U-test was, therefore, performed to identify differences between establishment samples, providing insight about shared common features and differences between samples; the null hypothesis expresses no differences between establishment samples, while alternative hypothesis indicates differences between them.

Finally, a principal component analysis (PCA) was carried out. This multivariate methodology is used to decompose the complex data matrix created with samples from each establishment, into a bilinear mathematical model, as well as to show patterns, clusters, trends and outliers of a sample set. To analyze variables from each establishment by PCA, samples were divided into three groups: the first one including macronutrients (*e.g.* starch or protein), the second one including metals and the third one considering temporary variations between samples.

3.5. Results and discussion

The combination of green chemistry and circular economy [19] has allowed the development of several pathways for FW recovery and valorization. The first step in every valorization process must include raw material description including chemical composition, biodegradability and statistical variability. However, nowadays, there is no specific international standard to apply to FW, but adaptation of methodologies to both raw material and process specific needs. This is of main importance for decision-making.

3.5.1. Samples description and discarding causes: typology and categorization

FW management problem might be solved through different strategies, from which prevention and post-valorization are two of the most important solutions known to date. Sample valorization, within the biorefinery concept, requires a prior detailed description of available raw material composition. The proportion of foodstuffs within each sample will determine its chemical composition and, therefore, potential valorization routes. Also, discarding causes for every analyzed food sample will determine necessary prevention actions to reduce the amount of generated FW[20], [12], [21].

Table 3-1. Food waste typology and discarding causes for each restaurant

Restaurant	Acronym	Food waste (FW) typology [12]	FW disposal categorization [13]
Italian restaurant	IR	Meat, bread, pizza, sauces, sweet desserts, egg, pasta and vegetable.	4
Fine dining restaurant	FDR	Fruit peels, vegetables, omelet, bread, sweet desserts, meat and bones.	1, 2
University campus cafeteria	UCC	Bread, meat, rice, potatoes, fruit, fish, sauces.	1, 4
Grill restaurant	GR	Meat and processed meat products, potato peels, mushrooms, other vegetables.	1, 2, 3

Table 1 describes, for each establishment included in the present study, a qualitative description of organic FW, that results in a typological description of FW according to each establishment. In general, a high presence of starchy FW, namely bread, pasta and rice, followed by vegetable and meat, was found. According to the categorization from [13] (see section 2.1.2) and to the above mentioned description of FW samples, Table 1 also includes the most probable causes of food discard found in every establishment.

In this sense, IR might be categorized as type 4 (FW related to excessive portion sizes) as main FW consisted of discarded portions of pizza, pasta and desserts. Other important FW from this kitchen were concentrated sauces (such as tomato or béchamel sauces) rich in oil, egg and flour. This type of restaurant represents a good candidate for special programs of preventive treatment in FW generation.

FDR includes buffet restaurant and special catering service for exceptional events (*e.g.* weddings) which fulfills requirements of type 2. It has also been included in type 1, due to strong seasonal impact on FW. Besides, crude fish and fruits were found in major proportion than other restaurants.

UCC FW might be included in the first and categories, as daily foresight is difficult to follow in this kind of restaurants, where customers -mainly students- have rotating schedules during the whole academic year (type 1). Meal portion size is also shown as a potential cause of food disposal (type 4), with special attention to bread portions, among the majority of waste found.

GR has meat dishes as main offer, which are considerably affected by type 3 of the proposed categorization: meat wastes were found both cooked and raw, the latter possibly discarded due to hygienic and quality parameters. FW found in these samples might be, therefore, the result of a combination between logistic control (types 1 and 2) and storage legal aspects (type 3). A relatively high amount of non-edible vegetables, such as peels, was also found among GR FW samples[9], [3].

3.5.2. Chemical composition of Food waste samples

According to [22], FW sample chemical composition is the base of any valorization process included in the biorefinery concept and will be discussed in detail in this section. As shown in

Figure 3, chemical profiles analyzed in this research depict a heterogeneous composition of FW, with a moisture content from 52.1 to 73.9% (w/w, wb). Remaining 27 to 47% (w/w, wb) is the range of all FW samples and is mainly composed of OF and minimal trace element fraction.

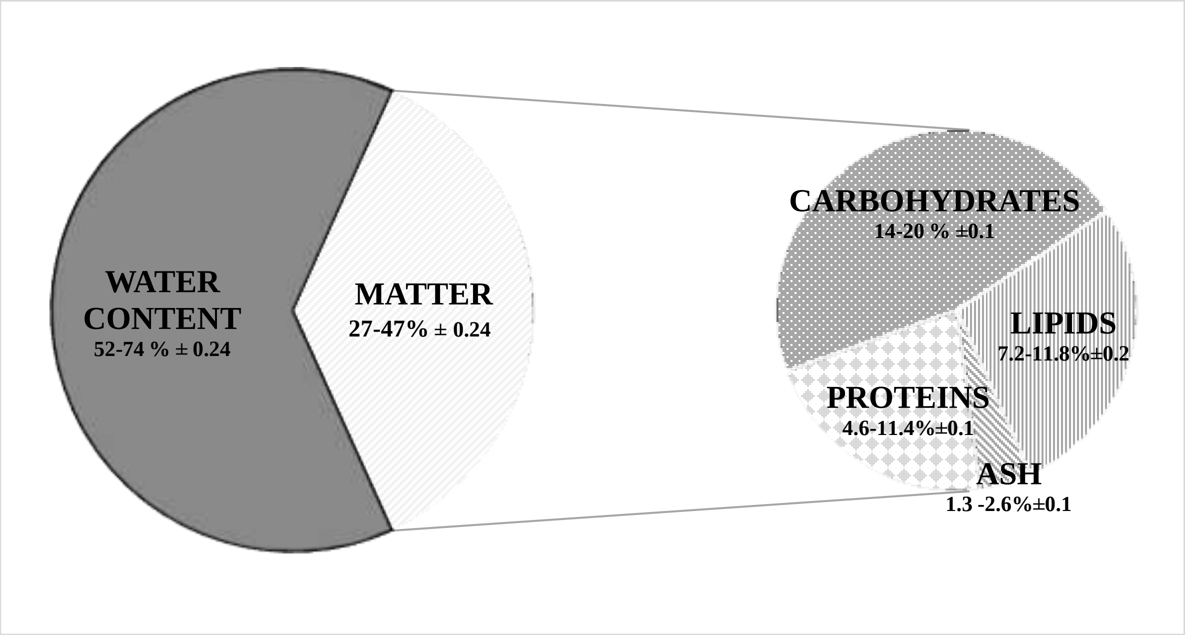


Figure 3-2. Range of concentration of components found in food waste samples on a wet basis

Humidity plays an important role in microorganism growth, enzyme activity and chemical reactions, such as *Maillard's*. There is a direct correlation between high humidity content, water activity and high biodegradability. High moisture content observed in FW samples considered in this study may has two effects. One effect may be the loss of nutrients in the draining process. In fact, due to high nutrient solubility, more than 74% of nutrients might be lost [23]. The second effect may be positively considered; initial humidity of FW may promote hydrolytic processes. Moreover, in terms of energy recovery efficiency, high moisture content reduces raw material lower calorific value. You et al. [24] reported values for lower heating values of SUR between 3,599.21 kJ/kg and 4,956.77 kJ/kg, while the heat minimum limit considered suitable for waste incineration is 3,344 kJ/kg.

Different criteria might be used to categorize the previously mentioned range of OF and trace elements present in FW samples; attending to its biodegradability, an easily biodegradable organic matter fraction (including proteins, lipids, simple sugars, starch) and a poorly biodegradable fraction (including fibre, i.e. lignin, cellulose and hemicellulose) could be defined. Moreover, to evaluate biochemical conversion potential, another classification may be adopted in accordance with the chemical composition of the dry matter fraction, resulting in four different groups: (1) carbohydrates, accounting for 14-20% (w/w, wb), this group being composed of simple sugar, starch and fibre; (2) lipids, representing 7.2 to 11.8 % (w/w, wb); (3) 4.6-11.4% (w/w, wb) proteins and (4) inorganic material, including ashes, accounting for the remaining 1.3-2.6% (w/w, wb).

3.5.3. Carbohydrates

Table 3-2Carbohydrate distribution in collected samples sorted by restaurant

Restaurant		**UCC[1]**	**FDR[2]**	**IR[3]**	**GR[4]**	**AVERAGE**
				% (w/w, db)		
Total carbohydrates		49.9	45.7	43.9	50.9	**47.4±3.3**
	Sugars	9.0±1.0	14.0±1.0	9.7±0.9	8.0±1.0	**10.7±2.2**
	Reducing sugars	3.1±0.2	3.8±0.3	2.5±0.2	2.4±0.4	**2.9±0.6**
	Starch	29.4±4.1	16.2±5.1	23.1±3.1	28.1±3.1	**24.1±6.1**
	Total fibre	8.4±0.8	11.7±0.6	8.2±0.3	12.4±0.8	**10.3±2.1**
Fibre types	**Cellulose**	3.6±0.4	4.3±0.3	3.5±0.4	4.1±0.5	**3.8±0.4**
	Hemicellulose	4.7±0.2	7.4±0.3	5.1±0.2	8.5±0.2	**6.4±2.2**
	Lignin	1.1±0.1	1.4±0.2	1.5±0.1	2.4±0.4	**1.5±0.1**
Cellulose/lignin ratio		3.2	2.9	2.4	1.64	**2.4 ±0.7**

(1) UCC: University campus cafeteria; (2) FDR: fine dining restaurant; (3) IR: Italian restaurant; (4) GR: grill restaurant

Table 2 shows carbohydrate distribution for every establishment considered in the present study. High deviation values are explained in section 3.3 (statistical analysis of FW samples). Carbohydrates, accounting for 47.4% (w/w, db) of the total biomass, were sorted out into different categories: simple sugars, reducing sugars, starch and fibre (including cellulose, hemicellulose and lignin). Starch represents the major component of the carbohydrate family, being 24.1% (w/w, db). Therefore, FW might be considered as “starchy waste” with a high valorization potential, i.e. bioethanol, microbial oil, bioplastic, etc.

Sugars represent an average of 10.7% (w/w, db) of total dry biomass of FW. The highest concentration of sugar was found in FDR samples (14%, w/w, db), followed by IR and UCC samples, with 9.7% (w/w, db) and 9% (w/w, db), respectively, and finally GR samples, with 8% (w/w, db). According to FW categorization presented in Table 1, it might be assumed that FW from restaurants offering fruits and sweet desserts in their menus will include higher concentrations of sugars.

Reducing sugars (RS) is a group of carbohydrates (including all monosaccharides and some di-, oligo- and poly-saccharides) which is able to act as a reducing agent. This group of sugars has the potential to reduce the biostability of organic FW, due to the presence of a free aldehyde or ketone group and may originate parallel reactions in recovery processes. An example of this type of reactions is the formation of N-glycoside bonds between reducing sugars and either proteins, peptides or amino acids. This reaction depends on the temperature, water activity and reaction time[12]. Reducing sugars represent an average of 2.9 % (w/w, db) of total dry biomass for all considered samples (Table 2). FDR samples showed a maximum value of 3.8% (w/w), followed by UCC with 3.1 % (w/w, db) and IR and GR, with values of 2.5 % (w/w, db) and 2.4 % (w/w, db), respectively.

Starch is a polysaccharide, composed of amylose and amylopectin, used as energy reserve in different plants, such as corn, potato, legumes, etc. It is stored in the form of granules within the cell organelles, known as amyloplasts. The average starch content of all dry samples (Table 2) is 24.1 % (w/w, db). Samples with higher starch content belong to UCC, 29.4% (w/w, db), while the minimum value was found for FDR samples, 16.2% (w/w, db), around two times below that of UCC.

As reported in Table 2, carbohydrates (sugars and starch) showed a joint average concentration of 34.8% (w/w, db). The presence of sugars and starch in FW may be valorized in different sectors, i.e. biotechnological industries or in environmental remediation[25],[19] . This kind of carbohydrates constitute a source of natural energy for microorganisms and are involved in many metabolic pathways. Easily assimilated carbohydrates are employed in anaerobic digestion, bioethanol production, lipid production, etc. [22, 26]. As an example, Kwan et al. [27] reported the use of *Aspergillus awamori* fungi to degrade the starch content from FW into glucose.

Fibres analyzed in this study were cellulose, hemicellulose and lignin, resulting in 10.3% (db) of the total dry biomass. This group of polysaccharides is included within dietary fibre, an element of plant cell walls with structural functions, which is present in vegetables, fruits and cereals. It may be transformed into simple sugars (e.g. glucose), yet through more complex treatments than those required for other polymers, such as starch [28]. To break fibre internal structure, current tendencies involve the use of an acid or basic chemical pretreatment, followed by an enzymatic treatment, as well as a thermophilic pretreatment. Other emergent pretreatments include different power sources, namely ultrasound or microwave energy, instead of the above mentioned chemical pretreatments, for the production of new biofuels, i.e. bio-oil [29].

Cellulose is a fibre composed of β-glucopyranosyl residue, connected by 1-4 linkages. It shows a semi-crystalline structure with amorphous gel regions, whose proportion depends on the moisture content. Samples of FDR and GR showed maximum values for cellulose content, 4.3 and 4.1% (w/w, db) respectively, followed by UCC, 3.6% (w/w, db) and IR, with 3.5% (w/w, db).

Hemicellulose is a cellulose with an internal structure modification; interspaces are occupied by carbohydrates, as xyloglucans (in *dicotyledoneae*), arabinoxylans (in *monodicotyledonaeae*) and β-glucans (in barley and oats). Average hemicellulose content for all samples is 6.4% (w/w, db); GR and FDR showed maximum values of 8.5 and 7.4% (w/w, db), respectively, while IR and UCC presented lower values than the average value.

Lignin is a heterogeneous and amorphous polymer, composed of monomeric units of phenyl propanoic acid. Maximum lignin value was found for GR, 2.4% (w/w, db), higher than the average value of 1.5 % (w/w, db) for all considered samples.

Finally, Table 2 also reports cellulose-to-lignin ratio, that describes fibre distribution. This parameter is used to evaluate biodegradability of FW samples [30]. It may also be used as a degradation indicator for FW during storage. Zheng et al. [31] reported a cellulose-to-lignin ratio close to 0.6 of samples from SUR, while data shown in Table 2 present values above 1.5 and a maximum value of 3.2 for UCC. This ratio depends on initial fibre concentration and it may be used to determine adequate pretreatment.

3.5.4. Lipids

Lipidic fraction, the second largest component of studied FW samples, reaches 28.1% (w/w, db) of the total dry biomass, and it presents a distinctive profile, as shown in Table 3. Maximum lipidic value was provided by FDR with 33.2% (w/w, db), followed by GR, UCC and IR with 27.3, 26.2 and 25.7 % (w/w, db), respectively.

Liquid and solid lipidic phases, representing an average of 20 and 81% (w/w, db) of all dry biomass, respectively, are closely linked to the typological composition of samples and, therefore, to the presence of meats, used frying oils, or sauces. Highest concentrations of solid lipidic fractions were found in GR and IR samples, being 29.9 and 23.4 % (w/w, db), respectively. According to Table 1, these are restaurants offering dishes rich in meat, fish and sauces. On the contrary, UCC and FDR showed higher values of liquid lipidic fraction,90.3 and 86.4 % (w/w, db), respectively. According to Table 1, most of their FW showed high presence of processed food, with the ability of accumulating oils during cooking processes. This typological difference represented 2.5 times the ratio between solid and liquid fractions in GR, and vice versa for FDR.

Fatty acid profile may help selecting valorization pathways in a biorefinery. Oleic acid (C18:1) is majority, with a solid/liquid phase average proportion of 45.8% (w/w, db). Both lipidic phases show a heterogeneous fatty acid profile, yet with important differences in linoleic (C18:2) and palmitic (16:0) acids content: linoleic acid is 1.63 times higher in the liquid fraction, while palmitic acid was 1.56 times higher in the solid fraction. These profiles may influence total unsaturation degree (TUD) and explain the presence of the above mentioned two phases: fraction with a TUD of 0.9 was liquid at room temperature, while solid fraction presented a TUD value of 0.7. Each fraction may be used in different routines in biorefineries. As an example, liquid fraction may be used in biodiesel production, while solid fraction, due to high viscosity, may be used in biolubricants production [22].

Table 3-3. Lipid content and fatty acid profile of food waste samples, based on matter state of aggregation.

Lipid fraction (%,w/w, db)			
RESTAURANT	**TOTAL**	**Liquid phase**	**Solid phase**
UCC[1]	26.2±1.2	86.5±1.8	13.5±0.1
FDR[2]	33.2 ±2.2	90.2±1.2	11.3±0.3
IR[3]	25.7±2.2	77.4±0.8	23.4±0.1
GR[4]	27.3±2.8	70.1±1.8	29.9±0.8
AVERAGE	**28.1±1.8**	**81.1±6.9**	**20±6.03**
Fatty acid profile			
Fatty acid (%, w/w, db)		**Liquid phase**	**Solid phase**
C8:0 (Caprylic acid)		--	0.2±0.1
C10:0 (Capric acid)		--	0.3±0.1
C12: 0 (Lauric acid)		1.7±0.1	0.2±0.02
C14: 0 (Myristic acid)		0.2±0.1	2.5±0.2
C16: 0 (Palmitic acid)		16.6±0.5	25.9±0.1
C16: 1 (Palmitoleic acid)		1.7±0.1	1.6± 0.1
C18: 0 (Stearic acid)		9.2±0.3	7.9±0.1
C18: 1 (Oleic acid)		45.0±1.9	46.5 ±0.1
C18: 2 (Linoleic acid)		23.0±1.1	14.1±0.1
C18: 3 (Linolenic acid)		1.1±0.1	--
Hydrocarbon chain properties			
LC[5]		17.3	17.1
TUD[6]		0.9	0.7

1) UCC: University campus cafeteria; (2) FDR: fine dining restaurant; (3) IR: Italian restaurant; (4) GR: grill restaurant

5: Length of chain, LC = Σ(n Cn)/100, where n is the number of carbon atoms of each fatty acid and *Cn* is the weight percentage of each fatty acid methyl ester.

6: Total unsaturation degree, TUD = (1 %MU + 2 %DU + 3 %TU)/100, where *%MU are monounsaturated methyl esters (%, w/w), %DU are diunsaturated methyl esters (%, w/w) and %TU are triunsaturated methyl esters (%, w/w).*

.Lipids in FW samples may also be divided in three different groups, according to their fatty acid chain length: (1) volatile fatty acids (VFA) with a number of carbons between 4 and 11, easily assimilable by microorganisms[30]; (2) medium chain fatty acids (MCFA) with 12-14 carbon atoms; and (3) long chain fatty acids (LCFA), with 14 to 24 carbon atoms in their molecules, showing an amphipathic character. The apolar chain of fatty acids, belonging to groups 2 and 3, is able to damage the cellular membrane of microorganisms. Moreover, LCFA (group 3) have the ability to generate vesicular structures, which may trap essential metabolites that are implicated in cellular metabolism and produce a negative effect in by-product yield[6]. According to data from Table 3, both lipidic phases present fatty acids belonging to the three above mentioned groups, yet with higher presence of MCFA and LCFA. Values of LCFA are similar in both phases (around 17) and only the solid phase presents more variability of VFA (*e.g.* C_8, C_{10}, C_{12}).

Fatty acids profile is directly related to FW typology, and therefore, to the presence of meat, fish or vegetables in FW samples. Meat and fish present more saturated fatty acids profiles, with higher tendency to be solid greases at room temperature; while vegetables and vegetable oils show higher unsaturation degrees, with higher tendency to be liquid. Lipidic profile reported in this study may be classified as LCFA-dominant and, therefore, highly suitable as raw material for biofuels, according to recent studies [32], [25].

3.5.5. Protein and nitrogen compounds

Proteins and nitrogen derived from FW management depict a high potential in biotechnology applications. Nutraceutical, cosmetic and pharmaceutical industries use protein compounds, such as collagen, chitosan, elastin or keratin. Moreover, some antioxidants are produced by hydrolysis of proteins, producing a peptide sequence with antioxidant properties. On the other hand, nitrogen plays a central role in microbial fermentation due to its implication in cell growth. To degrade protein to nitrogen assimilable by microorganisms, several applications have been developed. One example could be the use of filamentous fungi to produce proteolytic enzymes, which degrades the protein into free amino nitrogen (FAN) [33].

Processed foodstuff present in FW samples is subjected to many cooking processes such as frying, boiling or steaming. Moreover, part of the N present in protein molecules may be degraded by some microorganisms to other chemical species, such as NH_4^+ or NH_3[8]. Other forms of N that can be found in FW are nitrates and nitrites, both used as additives, helping to preserve the red color of meat and acting as antimicrobial agents [34]

Protein origin, according to the typological classification of tested FW samples, was mainly related to the presence of meat and fish dishes. Thus, even though many vegetables (such as soybean, lentils or chickpeas) are richer in proteins, they were not present in the evaluated samples. Average protein concentration for all FW samples is 20.6 % (w/w, db); GR was the establishment with largest amount of proteins, followed by FDR with a concentration slightly lower (23.5 and 21.5%, w/w, db respectively); IR and UCC samples presented a concentration below 20% (w/w, db).

Elemental or total nitrogen average for all FW samples is 3.5 % (w/w, db) with a maximum value of 3.9 % (w/w, db) for GR. Values for TN and TKN, reported in Table 4, allow to calculate that an average of 83.5%(w/w, db) of total nitrogen present in FW samples came from proteins. The largest difference between TN and TKN was found in samples from GR, with only a 78% (w/w, db) of nitrogen belonging to protein molecules. Nitrogen origin might be an important issue in biorefinery valorization processes. Thus, as an example, certain amounts of ammonium may have a negative effect on reaction yield in anaerobic digestion.

3.5.6. Elemental carbon content and C/N ratio

Elemental carbon is present in lipids, carbohydrates and proteins, with an average content of 52.5% (w/w, db) for all considered FW samples (Table 4). UCC and GR were the establishments with highest and lowest carbon content (55 and 50.2%, w/w db, respectively) as shown in Table 4.

C/N ratio is a parameter that allow getting information about OF from FW and may be used as an indicator in valorization processes. It is used as degradation indicator for biological material and also as reference value in bioprocesses (e.g. anaerobic digestion). It may help to determine the biodegradability of any biomaterial from two perspectives; on one hand, the extent of decomposition and, on the other hand, the capacity of the biomass to self-degrade within FW recovery chain [11]. Average C/N ratio for all FW samples is 14.9, with a maximum value of 16 for IR and a minimum one of 12.8, corresponding to GR.

Table 3-4. Nitrogen, carbon and ash content in food waste samples

RESTAURANTS	UCC[1]	FDR[2]	IR[3]	GR[4]	**AVERAGE**
	% (w/w, db)				
TN[5]	3.4±0.2	3.4±0.1	3.3±0.2	3.9±0.1	**3.5±0.2**
TKN	3.0±0.6	2.8±0.3	3.1±0.6	3.1±0.2	**2.9±0.1**
IN[6]	0.5±0.1	0.6±0.1	0.2 ±0.1	0.9±0.1	**0.6±0.1**
Elemental carbon	55.0±2	52.1± 0.3	53.0±0.2	50.2±0.1	**52.5±0.2**
Protein	18.3±0.2	21.5±1.5	19.5±0.2	23.5±2.1	**20.6±2.2**
C/N ratio	15.8	15.2	16.0	12.8	**14.9 ±1.4**
Ash	4.3±0.9	5.3±0.1	4.6±0.1	4.25±0.2	**4.5±0.3**

(1) UCC: University campus cafeteria; (2) FDR: fine dining restaurant; (3) IR: Italian restaurant; (4) GR: grill restaurant (5) TN: Total nitrogen (elemental); (6) IN: Inorganic nitrogen; (7) TKN: Total Kjeldahl Nitrogen.

In this study, the lowest C/N ratio from GR correlates with the highest value for elemental N and minimum elemental C value, due to high presence of meat and low quantity of food rich in carbohydrates (*e.g.* starch). However, C/N ratio provides limited information about nutrients proportion. As an example, UCC, FDR and IR samples presented similar C/N ratios (15.8, 15.2 and 16.0, respectively), however their nutrient profiles are quite different. UCC had a higher concentration of starch 29.4% (w/w, db), while FDR had higher concentration of carbohydrates, namely simple sugars (14%, w/w), lipids (33.2%, w/w, db) and fibres (11.7%, w/w, db). IR presented a homogeneous nutrient profile (see Tables 2-4). These are significant differences that may have important implications in FW recovery[22]. For example, fibre needs more difficult pre-treatments than starch. On the other hand, fibre may produce richer by-products, such as antioxidant.

3.5.7. Ash and trace element content

Ash content represents mineral and metal fraction present in organic samples and help understanding the mineralization process of FW. Gravimetric analysis revealed that ashes represent an average of 4.5 (w/w, db) for all FW samples (Table 4). Maximum value was 5.3% (w/w, db) from FDR, followed by IR with 4.6% (w/w, db), while GR and UCC presented values below the average.

A relationship between typological classification of FW samples and ash content is not easy to elucidate. Thus, every foodstuff shows a different mineral profile. To study minerals present in FW samples, they were sorted in three categories, according to their concentration level, namely main elements, trace elements and ultra-trace elements [35].

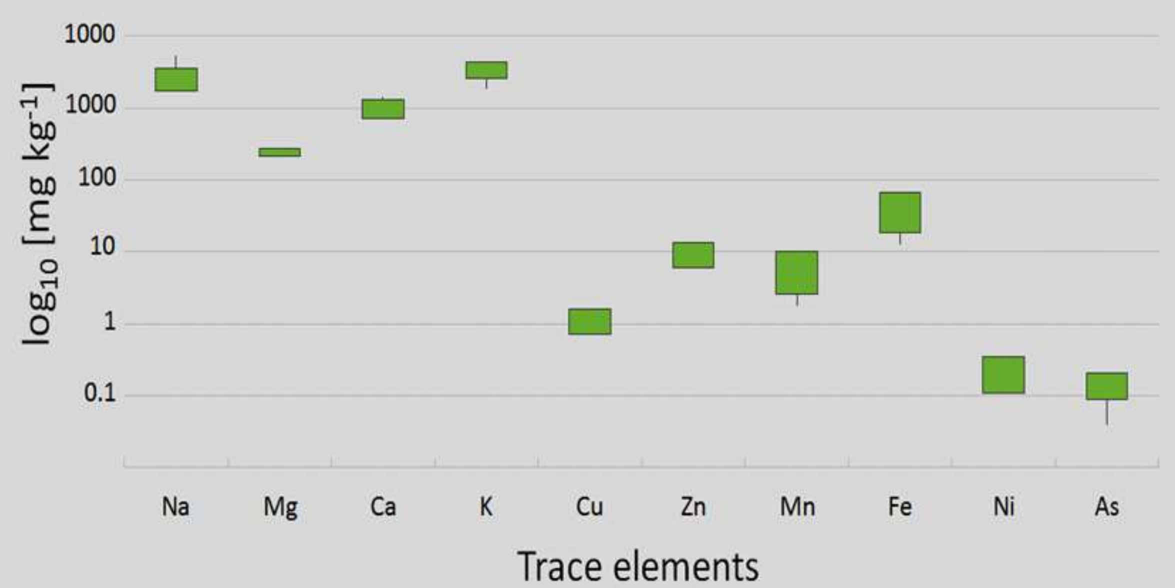


Figure 3-3. Trace element concentration in food waste samples

Three well-defined measure scales, according to these ranges of concentration, may be appreciated in Figure 4. The first group of minerals, including Na, K, Ca and Mg, represent in average 0.32% (w/w, db), 0.26% (w/w, db), 0.11% (w/w, db) and 0.03 % (w/w, db) of the total biomass on a dry basis, respectively, and may be considered as main components of the metal group (high range of ppm). The second group is composed of Zn, Mn and Fe; their values (8.01, 3.76 and 26.15 mg/kg, respectively) are in the low range of ppm and can be considered as trace elements. The third group is considered in the range of ultra-trace elements and is composed of heavy metals, namely Cu, Ni and As (0.9, 0.21 and 0.1 mg/kg, respectively). Their concentration values are below 1 ppm, as well as Pb and Cd, that have not been represented in Table 4 as their concentration were lower than the detection limit. Heavy metal concentration in FW samples is less than the maximum value allowed by the European directive 199/31/EC.

Trace elements are implicated in synergistic and inhibitor routes in microorganism fermentations [36] through metabolic pathways, enzymatic stabilizations and cofactors. It has been estimated that to produce 1 kg of biodiesel from microalgae *Auxenochlorella protothecoides,* 0.03 kg of magnesium and 0.06 kg of potassium are required. The importance of Mg, Fe, Zn, Cu and Mn in lipogenesis has been reported for concentrations of 1.5g/l (Mg) and 0.001 g/l (Fe, Zn, Cu or Mn) [37].

In anaerobic digestions, metals like Fe and Ca, may help stabilizing the bioprocess; also, Fe has a significant role in cell redox homeostasis due to its ability to transport electrons and act as a buffer [3]. On the other hand, Ca is a cofactor that helps to capture LCFA, thus reducing the above mentioned negative impact of these fatty acids [38]. Nevertheless, in a valorization process, bioaccessibility of mineral nutrients must be considered, taking into account that metal solubility might be reduced as the result of interactions with substances such as phytates, oxalates, tannins and fibres. On the other hand, cooking processes may improve mineral bioaccessibility, although each one exhibits different behavior, so this assessment may not be a general rule. All samples analyzed in this study showed similar concentration ranges to those reported in literature.

3.6. Statistical analysis of Food waste samples

Statistical tools, i.e. dispersion and distribution analysis or principal component analysis (PCA), were used to highlight similarities and differences between samples from different establishments. Statistical methodology allows the integration of typology profile, physical and chemical properties and biodegradability information. This methodology influences the decision-making process in the valorization of the waste. Fisgativa et al [8] emonstrated this impact in predictions of FW behavior in anaerobic digestion processes. The present statistical study may help predicting development of future valorization processes.

To provide insight about shared common features and differences between samples, four different tests were carried out. Considered data show a high dispersion; also, skewness and kuortosis values indicate a non-normal distribution. Furthermore, the Kolmogorov-Simonov test confirmed asymmetric distribution of each parameter with the exception of fibres. As a result, it was necessary to use the U Mann-Whitney test (a non-parametric analysis), used to evaluate the differences between restaurant samples. Finally, PCA showed clusters of samples and their distribution according to composition. Table 5 and Figures 5-7 summarize both dispersion and distribution of all considered samples, showing their inter- and intra-variabilities.

Table 3-5. Statistical test for macronutrients and trace elements considering variability provided by each establishmen

ELEMENTS AND MACRONUTRIENTS	**Dispersion**		**Distribution**		**Normality tests**	**Non-parametric analysis**
	Average value (%, w/w)	CV (%)	Skewness	Kurtois	K-V[1] p ≤ 0.05	U Mann-Whitney p≤ 0.05
MACRONUTRIENTS						
Ash (%, w/w)	4.5	30	2.2	12.3	≥ 0.001	Low variability IR ≠ GR, UCC
Protein (%, w/w)	20.6	40	0.2	0.1	≥ 0.001	Low variability GR ≠ FDR, IR, UCC
Simple sugar (%, w/w)	10.7	30	1.3	3	≥ 0.001	Low variability FDR
Starch (%, w/w)	24.2	33	-0.4	-0.7	≥ 0.001	High variability UCC ≠ FDR ≠ IR ≠ GR
FND (%, w/w)	10.1	40	0.8	0.1	0.20	Low variability FDR ≠ IR, GR, UCC
Lipids (%, w/w)	28	20	0.4	1.2	0.07	No differences
ELEMENTS						
C (%, w/w)	53	10	1.3	6.7	0.019	Low variability UCC
N (%, w/w)	3.5	15	1.2	2.5	≥ 0.001	No differences
Na (ppm)	3282.9	30	0.2	-0.9	≥ 0.001	High variability
K (ppm)	2620.6	40	1.2	0.7	≥ 0.001	High variability
Mg (ppm)	228.2	30	4.8	30.2	≥ 0.001	No differences
Ca (ppm)	1167	2	5.1	29.5	≥ 0.001	High variability
Cu (ppm)	1	35	3.2	16.2	≥ 0.001	High variability UCC = GR
Zn (ppm)	8.1	35	1.7	3.7	≥ 0.001	High variability UCC = FDR = GR
Mn (ppm)	3.7	33	7.5	58.5	≥ 0.001	Variability UCC = FDR = GR
Fe (ppm)	26.15	5	3.2	13.8	≥ 0.001	High variability UCC = IR
Ni (ppm)	0.21	30	6.1	42.3	≥ 0.001	Variability UCC = FDR = IR
As (ppm)	0.1	30	6.7	47.6	≥ 0.001	Variability UCC = GR = IR

UCC: University campus cafeteria. FDR: fine dining restaurant IR: Italian restaurant GR: grill restaurant

Sample dispersion degree was studied through the coefficient of variation (CV). Overall data showed high values of CV, which means that reported values were very dispersed due to internal factors (i.e. chemical matrix or typological distribution), as well as external factors, i.e. analytical methodologies. According to Table 5, within this group of nutrients, total fibre and protein presented the greatest dispersion (CV equal to 40%) followed by starch (CV equal to 33%), simple sugar (CV of 30%) and ash. However, high dispersion is not reflected in elemental carbon dispersion, with a CV of 10%. This value, together with CV calculated for elemental N (15%) indicate that C/N ratio is not influenced by variations of composition in studied FW samples. Nevertheless, C/N ratio may not show the real potential for efficient valorization, because it does not differentiate between raw material components. Variability is an essential parameter to consider, when seeking for an efficient valorization pathway. An example that contributes to the variability study was found in the protein content related to proportion of meat in FW samples, that was not reflected in variation of N values. As a conclusion, this variability in protein content my not be translated into elemental nitrogen variability, thus there are other nitrogenated chemical compounds (i.e. nitrites, amino acids or ammonium) that also participate of C/N ratio. Finally, both ash content and trace element CV reported a value equal to or slightly higher than 30%, with the exception of iron and calcium (Table 5). Variability of these elements is correlated to important differences in typological composition and how a certain food was made. For example, food derived from milk will have a higher amount of calcium. While manufactured food depicts higher Na and K concentration that non-processed food.

With the objective of understanding sample variation, a principal component analysis (PCA) was carried out from three different points of view [21]. Figure 5 shows the three principal macronutrient components that explain 70% of sample variability.

PC1 (*x*-axis) explains the distribution of restaurant samples as a function of protein and ash content. In this case, important variation between sample restaurants was observed; thus only GR showed more dispersed samples. PC2, corresponding to *z*-axis, explains the distribution of polymers, i.e. starch, fibres or lipids. UCC showed less dispersion than other restaurants, while GR and FDR depicted the highest dispersion. PC3 (*y*-axis) reflects simple sugars distribution and shows maximal dispersion for FDR samples.

PCA allows to analyze samples in a collective way, besides visualizing typology differences. As an example, protein and ash patrons were homogeneous in all restaurants with the exception of GR, that serves meat specialties. Besides, simple sugars show higher dispersion in FDR (menu includes a high amount of sweet foodstuff and fruits) than in the rest of establishments. As a general rule, it might be assumed that the more specialized the restaurant, the higher the dispersion of one component, in relation to the other restaurants.

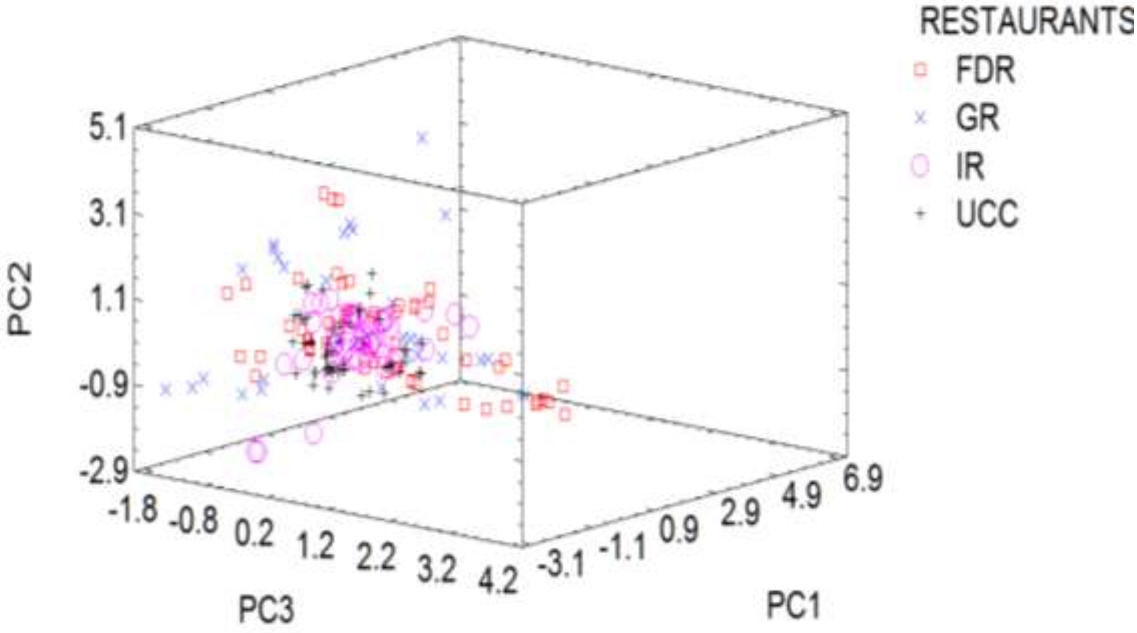


Figure 3-4. Principal component analysis for macronutrients for different restaurant food waste[1].

The U Mann-Whitney test allows to find out similarities and differences between sample restaurants, in terms of chemical composition. Data shown in Table 5 confirm the first PCA result about low variability for ash and protein, while the U Mann-Whitney test was more specific and indicated both that IR showed higher ash content variability and that GR exhibited the highest protein dispersion degree. Simple sugars also presented low variability between establishments and confirmed the specific pattern of FDR, elucidated from PCA, while lipids did not present significant differences between restaurants. Finally, starch dispersion showed in PCA was confirmed by the U Mann-Whitney test, being all restaurants statistically different.

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[1] UCC: University campus cafeteria. FDR: fine dining restaurant IR: Italian restaurant GR: grill restaurant

As mentioned above, metals (shown in Figure 6 and Table 5) are presented in three different scales of concentration. Trace element PCA explained more than 70% of variation of this group of nutrients. In this PCA, PC1 (*x*-axes) includes main metallic elements, namely Na, Mg and Ca, without important variations, with the exception of outliers. PC2 (*z*-axes) shows trace element (K, Zn, Mg and Fe) variation, with concentrations close to ppm and ppb. GR samples presented higher variability in PC2 with respect to other establishments, showing a cluster that has a correlation between meat presence and iron concentration from myoglobin, that was corroborated by the U test. Finally, PC3 (*y*-axes) described variations of ultra-trace metallic elements, with concentrations close to ppb. In this case, no significant variations were found, and the U Mann-Whitney test only presented partial variations between restaurants, as reported in Table 5.

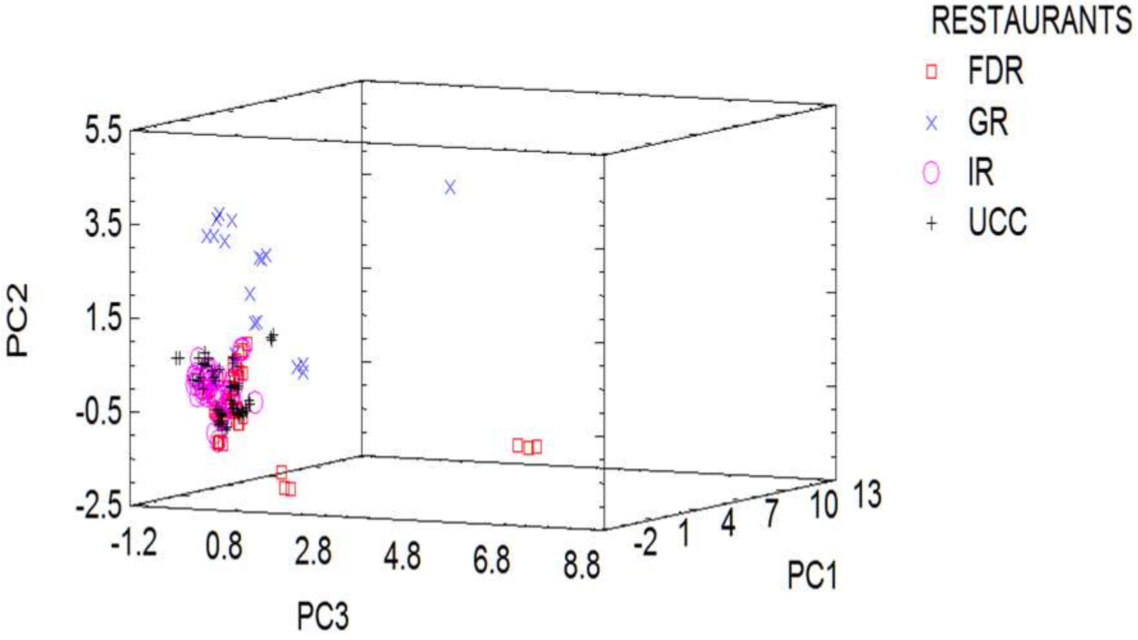

Figure 3-5. Principal component analysis for metal content in different restaurant food waste[2].

A final PCA to consider time variations was carried out and results are shown in Figure 7. As mentioned in “Materials and Methods” section, food waste samples were collected during five months, covering two seasons (winter and spring) aiming to analyze time variations. The

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[2] UCC: University campus cafeteria. FDR: fine dining restaurant IR: Italian restaurant GR: grill restaurant

most significant variation was found in PC2 (*z*-axis) that shows protein variations and PC1 (*y*-axis) that exhibits variations in simple sugars over time. Finally, PC3 (*x*-axis) explains starch variations, showing significant monthly deviations. Data show not only typology composition issues, but also reports information about user eating habits.

In this study, statistical analysis shows high correlation between typology and chemical composition. PCA shows high degree of sensitivity, while detecting small changes in patterns, such as protein variability in GR and special FDR pattern. PCA also shows metal variability and confirms concentration scales and seasonal differences between components. These results are in agreement with all statistical tests.

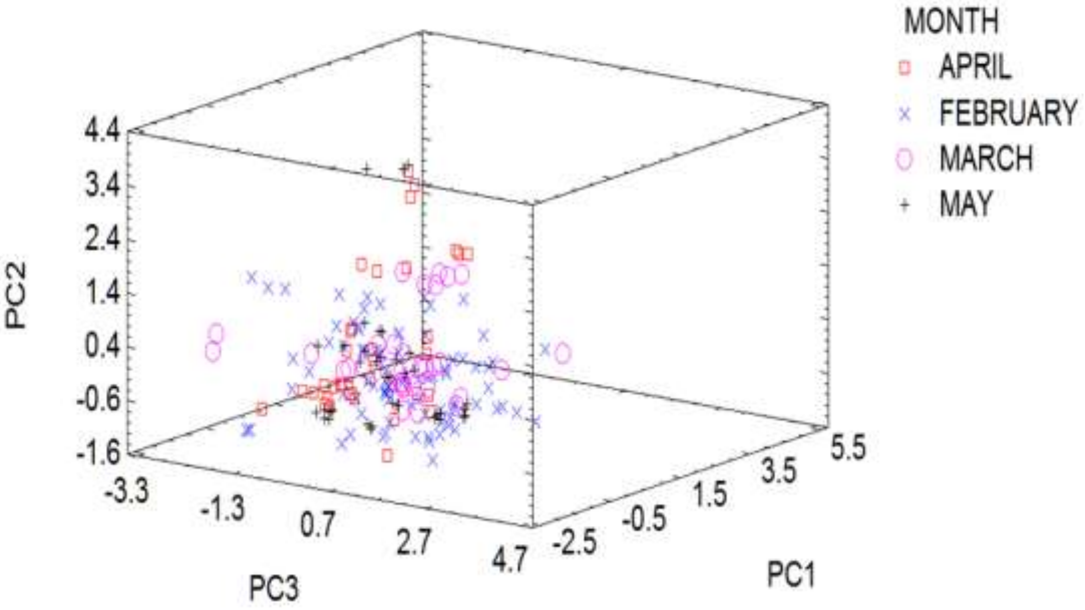

Figure 3-6. Principal component analysis along the year in which sampling was carried out.[3]

An interesting result reported in this study, in agreement to that reported by Fisgativa et al. [17], is the high CV, that PCA analysis cannot explain beyond 70%. High CV values indicate that mean includes dispersed values that might be considered as errors. As a result, to achieve statistically reliable conclusions, mean cannot be used. However, considering food waste case, variations in typology may partly explain sample deviation, while other factors, i.e. seasonal

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[3] UCC: University campus cafeteria. FDR: fine dining restaurant IR: Italian restaurant GR: grill restaurant.

variations, complex chemical matrices or non-specific analysis, may explain the remaining deviation.

In each country, FW is regulated by a specific directive. The current standard "*RSB EU RES standard for certification of biofuels based on waste and residues*" describes how to characterize this biomass. Several tests, including elemental analysis, calorific value or humidity tests, are described by this standard. Nevertheless, this standard does not include a chemical component test. And this is unfortunate, as part of the unexplained variability may originate in different interactions within the chemical matrix. Carmona et al. [22] reported several interactions in FW matrix, i.e. amino acid reactions carried out by the Maillard reaction, redox reactivity from reducing sugars and fibre degradation from pre-treatment.

Conclusions

Combination of typological analysis, chemical characterization and statistical tests could provide important information for FW valorization via bioprocesses. Qualitative analysis of hospitality FW typology profile showed an elevated presence of rich-in-starch foodstuff. FW chemical profile showed high moisture content and high degradation potential of organic fraction. Statistical analysis reported differences in restaurant-to-restaurant profile and showed a complex matrix. In this study, non-normality distribution shows the importance of developing a specific standard model for FW analysis. Chemometric tools could play an important role in chemical analysis. Proposed methodology provides a good starting point to set up the basis of FW biorefinery

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CHAPTER 4.

TRANSESTERIFICATION OF THE LIPID FRACTION FROM SOLID FOOD WASTE

4.1 VALORIZATION OF FOOD WASTE FROM RESTAURANTS BY TRANSESTERIFICATION OF THE LIPID FRACTION

Abstract

Food waste contributes to increase the environmental impact, besides the ethical issue. One interesting way of valorization is its conversion in biofuel, thus helping to boost the concept of circular economy. The target of this work was to find out the feasibility of the use of the oil included in solid food waste (SFW) to produce biodiesel that meets the European biodiesel standard EN 14214. For this purpose, Soxhlet extraction of the lipid fraction of SFW from different restaurants has been carried out. Fatty acid composition was analyzed and potential differences concerning the source of SFW were evaluated through principal component analysis. Results showed significant differences in the oil fatty acid composition depending on the restaurant. However, oil physical and chemical properties were similar, excepting the acid value. Due to the high free fatty acid content (acidity of 11.21 mg KOH/g) of the oil from fine dining restaurant residues, acid-catalyzed esterification pre-treatment to the alkaline transesterification was needed. The fatty acid composition of oils from SFW differs depending on the restaurant, but the range of fatty acid methyl esters (FAME) is similar to that found in vegetable oils, showing a content of oleic acid (C18:1) between 36.39-41.57% w/w and linolenic acid (C18:2) of 21.37-38.63% w/w. Several chemical and physical properties of SFW oil biodiesel were analyzed. It was found that biodiesel fulfil the European standard EN 14214, with the exception of FAME yield, oxidation stability and glyceride content. For this reason and to improve biodiesel quality, further reaction optimization study, blending with diesel fuel or the use of additives is strongly recommended. It may be concluded, from this field trial, that oil from SFW from different restaurants may be mixed together and used to produce biodiesel. To corroborate this statement, further diesel engine tests are needed.

4.1.1 Introduction

Solid food waste (SFW) comprises food residues and is composed of processed food or discarded edible raw materials [1]. Recently, FAO has reported that more than 50% of the food produced is discarded, reaching over 1.3 billion tons of wasted food per year [2]. EUROSTAT data from 2006 show an annual food waste generation in EU27 of 89 Mt, equivalent to 179 kg per capita. Considering EU population growth by 2020, a production of SFW by about 126 Mt is expected, corresponding to an annual CO_2 related emissions of 240 Mt [1]. These figures demonstrate that SFW has a great impact on environment, food quality, safety and security; a sustainable management of SFW represents a challenge from an economic and ecological point of view. Furthermore, food waste involves a relevant ethical issue, as while food is wasted by developed countries, one billion people die of starvation in the rest of the world [3]. Different sources of SFW production have been identified [1]:

- Manufacturing sector
- Household sector
- Wholesale/retail sector
- Food service sector

In the EU27, 14% of total SFW is provided by the food service sector, reaching 12.3 Mt (an average of 25 kg per capita). Also, there is a significant discrepancy when either EU12 or EU15 is considered, as SFW from food service sector provides 12 kg or 28 kg SFW per capita, respectively. SFW production from food services is caused by inadequate storage, technical malfunction while food is processed in the food chain supply, food safety regulations or excess of food *vs.* request [3].

The composition of food waste from food service is very heterogeneous and depends on the establishment. It is mainly collected from restaurants, food markets (grocery stores), hotels, cafeterias, caterings and bakeries. Food waste includes different mixtures of food, i.e. meat, vegetable, carbohydrate (rice, potato and bakery products, as donuts and bread), fish, legumes, etc.

The planet is involved in a climate crisis at both global and local level. A change in the energy model, besides the selection of new feedstock and development of efficient conversion technologies to reduce greenhouse gas emissions is needed [4]. In this sense, different protocols as the one in Montreal (1990) or Kyoto (1997) contain legally binding commitments. The EU has developed a strategy for 2020 that includes three objectives, called “20-20-20”, in order to reduce greenhouse gas emissions (GHG), to increase renewable energy in final energy consumption and energy efficiency.

In the EU, the industry is the sector that generates the highest amount of exhaust emissions (58%, that is 2.6 Mt) followed by transport (20%, 1.02 Mt). Waste management (including SFW) represents 4% of the total emissions. In this context, increasing the use of alternative fuels is key to reduce GHG emissions. Considering transport, liquid biofuels should have a relevant role. Although, in 2014, 64.1% of produced renewable energy originated from biomass, liquid biofuels for transport only represented 6% of the overall share of renewable energy. At this point in time, SFW is an interesting raw material that may be transformed into biofuels, besides high value-added products through the biorefinery concept. Chemically, food establishments contribute to SFW with lipids, carbohydrates, amino acids, phosphates, vitamins and other types of carbon; the lipid fraction can be converted into biodiesel or fatty acid methyl esters (FAME) by transesterification. For this purpose, a previous extraction of lipids from SFW is needed. This operation can be carried out using a conventional Soxhlet or through supercritical extraction [5].

Before FAME from SFW is produced, is key to analyze the acid value (AV) and moisture content of extracted lipids. In this sense, alkali-catalyzed transesterification using KOH is preferred when triglycerides with low free fatty acid (FFA) content are used. For high FFA feedstock (above 3%), two-step reaction is recommended, combining acid and basic catalyzed transesterification [6, 7]. The necessary amount of KOH is supplemented with the necessary amount of catalyst in a second-step transesterification, to neutralize the oil acidity. Also, Thiruvengadaravi et al. [8] proposed an acid esterification pretreatment to reduce AV to 1.3 mg KOH/g in the oil. Yang et al. [9] achieved a biodiesel conversion rate of 98.5% and 97.8% from waste noodles, using both acid and basic catalysts, respectively. Moreover, the viability of biodiesel production from lipid obtained from fungal hydrolysis of SFW was demonstrated in a recent study by Karmee et al. [10]. Authors extracted the lipid fraction with hexane and diethyl ether, after mixing SFW with water, achieving a FAME yield close to 100%.

Biodiesel quality is regulated by the European standard EN 14214. In this sense, several studies [11-15] have demonstrated that the most important physical and chemical properties of biodiesel depend on the fatty acid composition of triglycerides used as feedstock, which constitute the lipid fraction of SFW. However, due to complex thermochemical processes involved during cooking and storage of food, SFW compounds content can undergo several changes. For example, complex structures such as colloidal dispersions and emulsions may take place, while lipids can interact with proteins or sugars [16]. Tree types of reactions are involved during food frying, namely thermolytic, oxidation and hydrolytic processes [17]. Moreover, during storage, unsaturated fatty acids can be oxidized. For these reasons, to optimize biodiesel production in a SFW-based biorefinery, an exhaustive characterization of the lipid fraction is imperative.

In this study, to characterize triglyceride content and fatty acid composition of SFW from different restaurants from Córdoba (South of Spain), a previous Soxhlet extraction of the lipid fraction has been carried out. Then, biodiesel has been produced and its quality has been evaluated, thus increasing the added value of the proposed biorefinery from SFW from food service sector. The aim was to produce high quality biodiesel and to evaluate significant differences depending on the source of S

4.1.2 Material and methods

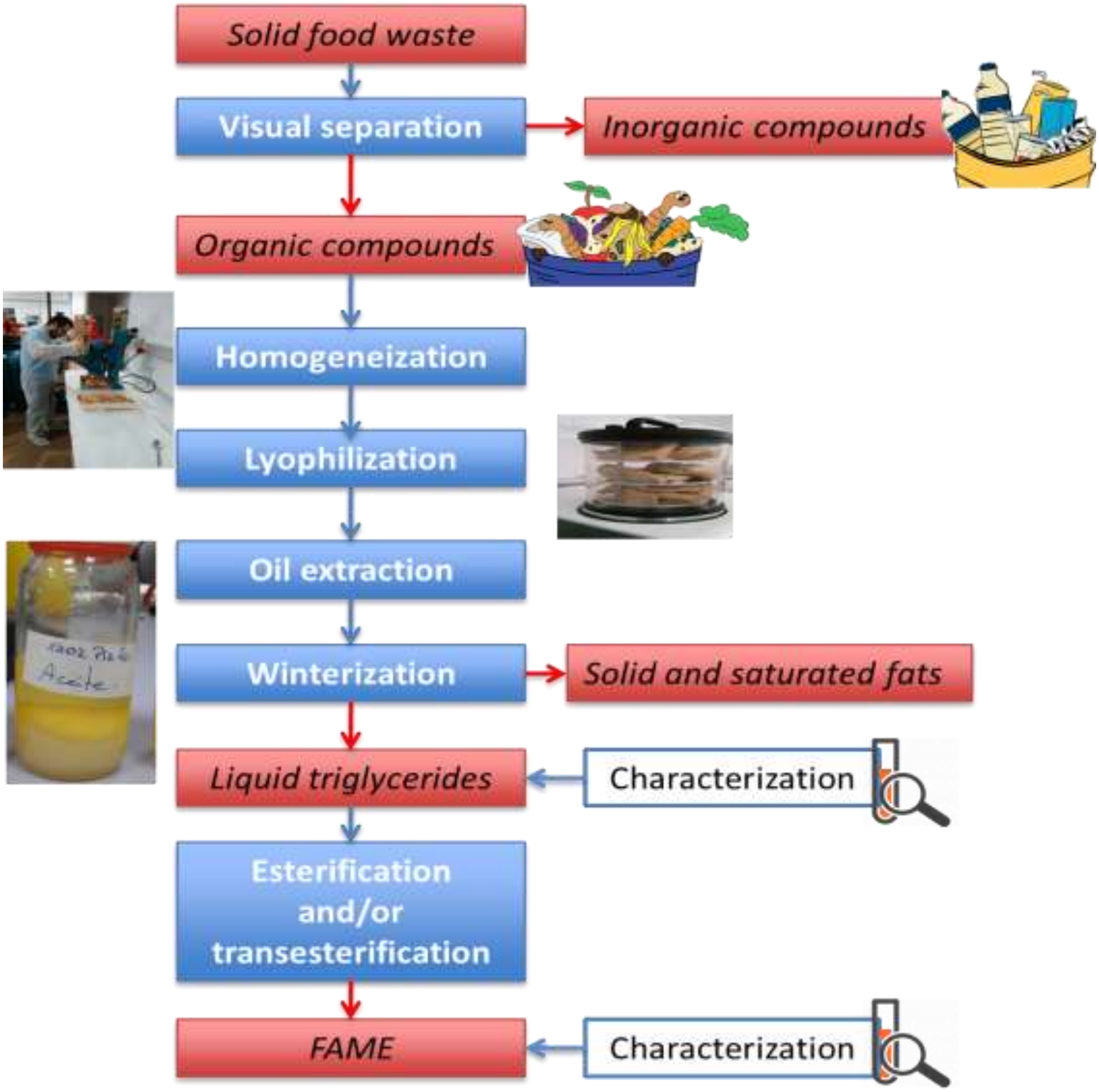

Figure 4.1-1 experimental layout for biodiesel production

4.1.2.1 Sampling and preparation of raw materials

A set of 30 samples of SFW from three different restaurants, that is, an Italian restaurant (IR), a fine dining restaurant (FDR) and a University campus cafeteria (UCC) was analyzed and processed as described in Figure 1. With the aim to evaluate the variability between samples and restaurant, sampling was conducted on random days during four months. To homogenize each sample, inorganic residues such as cans or plastics were discarded, for subsequent milling the organic fraction using a pin mill. Finally, each sample was lyophilized for three days in a lyophilizer LyoQuest model Telstar 55 (Terrassa, Spain) and stored at 4°C until further analysis. This procedure preserves organic material from microbial contamination, thus enhancing lipids extraction and further solid matrix processing.

4.1.2.2 Oil extraction and characterization

Sample oil extraction was carried out by the Sohxlet method, in a 500 mL Sohxlet apparatus, recirculating hexane for analysis CODEX (95% purity, J.T. Baker, from Merck) during one hour. Finally, hexane was removed by rotary evaporation under vacuum at 65°C, until complete removal of hexane. Subsequently, oil content on a dry basis was calculated. To reduce the content of saturated fatty acids, a winterization process was performed using a temperature-control centrifuge (MPW-351R, Poland) during 10 min, at 0°C and 2000 rpm. Moreover, the content of water in the oil, □□ was analyzed using a Karl Fischer titrator, model Mettler Toledo DL 32. The oil AV or free fatty acid content was analyzed following the standard EN ISO 660. Kinematic viscosity, □, was determined using a glass capillary-type viscometer (Cannon-Fenske, size 200) following the standard EN ISO 3104.

4.1.2.3 Transesterification process

As previously mentioned, depending on the oil acid value [8, 18], one- or two-step transesterification process may be needed. In the present work, for oils with acidity lower than 5 mg KOH/g, an alkali-catalyzed transesterification was carried out. In the present work, acid pre-esterification was only needed in case of FDR and IR samples. It was carried out using 1 % (w/w) H_2SO_4 [96% purity p.a (Pa-ISO from Panreac)] and 8:1 methanol/oil molar ratio using methanol (ACS–ISO from Panreac). Reaction was carried out at 65°C during two hours, in a 50 mL three-necked flask equipped with reflux condenser to prevent evaporation of methanol. Flask was immersed in a bath with temperature control and magnetic stirrer. For low acidity oils, alkali-catalyzed reaction was carried out using 1.5 (% w/w) KOH pellets [85 % p.a., CODEX (USP-NF)] and 6:1

methanol/oil molar ratio (methanol ACS–ISO from Panreac), at 65°C during one hour of reaction.

Subsequently, samples were centrifuged at 5000 rpm to separate glycerol from the ester phase. The centrifuge used was a refrigerated MPW-351R, from MPW Med. instruments (Poland). Then, they were washed two times with distilled water, dried with anhydrous Na_2SO_4 [99.5 % p.a., CODEX (RFP, USP, BP, Ph. from Panreac)] and subsequently, filtered, as explained in [4].

4.1.2.4 Biodiesel properties analysis

FAME yield was determined by gas chromatography, following standard EN 14103. A Perkin Elmer gas chromatograph Clarus 500 (Waltham, Massachusetts, USA) coupled to a flame ionization detector (FID) equipped with a Perkin Elmer capillary column 30 m × 0.25 mm Elite 5-ms (0.25 µm particle diameter) was used. For the oxidation stability analysis, 893 professional biodiesel Rancimat from Metrohm (Herisau, Switzerland) was used, following EN 14112 normative. The determination of carbon residue (CR) was carried out following ASTM D-4530 using a micro carbon residue tester (Alcor MCRT-160 by PAC, Houston, US).

For mono-, di- and triglyceride (MG, DG and TG, respectively) and glycerol determination, UNE-EN 14105 was followed, and methanol (ACS–ISO from Panreac), n-heptane for analysis CODEX (reag, Ph, Eur from Merck) and piridine [99.8% p.a. CODEX (Reag, Ph, Eur)] were used. 1,2,4-butanetriol purity ≥ 90% p.a. (from Sigma-Aldrich) and 1,2,3-tricaprin solution 8000µg/ml in pyridine analytical standard p.a. (ASTM D6584, from Sigma-Aldrich) were used as internal standard. N-Methyl-N-Trimethylsilyl (trifluoroacetamide) purity ≥ 98.5 % p.a. (from Sigma-Aldrich) was used for GC derivatization. Reagents were supplied by Sigma–Aldrich.

Once variability between samples was studied, provided the small amount of each SFW sample, therefore including a reduced amount of oil, kinematic viscosity (□), density (□), high calorific value (HCV), flashpoint (FP), cold filter plugging point (CFPP) and water content (□) were determined, for each restaurant, by blending all oils collected during the 4-month sampling. In any case, the idea is to find out whether all residues from each restaurant may provide suitable residual oil to be converted in biodiesel. HCV was analyzed following the standard ASTM D240, by means of an IKA calorimeter bomb C200 (Satufen, Germany). CFPP was analyzed using a cold filter plugging point HCO 842 (Herzog by Pac) equipment, following the ASTM D 6371 standard. Density was analyzed following EN ISO 3675 standard. Flash point was monitored (according to EN ISO 2719) by Seta Flash series 3 plus, from Instrumentación Analítica S.A. (Madrid, Spain). Water content was measured following the same procedure mentioned in section 2.2. Cetane number (CN) was predicted using the mathematical equation (1):

$CN = \sum X_{ME}\,(\%\ w/w)\ CN_{ME}$

Where X_{ME} is the weight, in percentage, of each methyl ester, and CN_{ME} is the cetane number of individual methyl esters [15].

To determine FAME fatty acid composition, 5 ml of hexane were added to 0.1 g of FAME and stirred 30 s with a vortex. Subsequently, 0.5 ml of sodium methylate was added and stirred with a vortex for 3 min. Finally, the mixture was centrifuged (500 rpm) and the upper phase was selected. Experiments and analysis were carried out in triplicate.

2.5. Statistical analysis and predictive models

Statistical analysis was performed using IBM SPSS Statistics 22 (NY, US). Results were expressed as mean value ± standard error. ANOVA, Tukey LSD test and principal component analysis (PCA) were used to assess the differences between oils present in SFW from each restaurant.

4.1.3 Results and discussion

4.1.3.1 Solid food waste oil content and properties

Results concerning lipid fraction included in SFW samples are shown in Table 1. The highest lipid content was provided by SFW samples from FDR (33.24% w/w) and showed significant statistical differences (underlined figures, P = 0.047) compared with UCC (26.34 % w/w) and IR (26.13% w/w). This lipid fraction provides a fatty acid composition similar to that from other potential oleaginous feedstocks for biodiesel production. Regarding non-edible oleaginous sources, oil contents of 27, 38 and 32% w/w for *Pongamia pinnata*, *Camelina sativa* and *Cynara cardunculus* [19], respectively, have been reported.

It is important to mention that standard deviation show both variability between restaurants, besides statistical differences between samples from the same restaurant, as depicted in Table 1. In this sense, it may be seen the huge variability of samples, considering solid fats in all restaurants, water content from UCC samples or acid value from IR and FDR samples. This is due to the variability of the meals depending on the day, season and consumers.

Table 4.1-1. Oil properties from solid organic food waste (SFW) for each analyzed restaurant[1].

Properties	Restaurant			ANOVA	
	IR	FDR	UCC	P-value	LSD test
Lipid content (% w/w)	26.13 ± 2.29	33.24 ± 2.71	26.34 ± 1.30	0.047	FDR vs IR-UCC
Solid fats at 0°C (% v/v)	18.63 ± 4.78	9.30 ± 3.37	11.05 ± 3.53	0.224	--
Water content, ϑ (mg/kg)	430.00 ± 13.81	290.14 ± 52.30	358.79 ± 110.30	0.654	--
Kinematic viscosity, ν, at 40°C (mm^2/s)	15.26 ± 1.09	16.13 ± 1.21	15.05 ± 1.50	0.822	--
Acid value, AV (mg KOH/g)	5.27 ± 2.89	11.21 ± 3.37	2.10 ± 0.91^b	0.060	FDR vs UCC

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[1] IR: Italian restaurant IR, FDR: fine dining restaurant, UCC: University campus cafeteria

SFW samples comprise solid fat (saturated fatty acids) in a range between 9-18% v/v oil, so its extraction would significantly improve biodiesel cold behavior properties. Also, it may be seen from Table 1 that SFW oils show a kinematic viscosity in the range between 15-16 mm^2/s, values frequently found in oils used as raw material for biodiesel production.

High values of acidity and water content (ϑ) in oils may promote parallel reactions during transesterification (such as saponification [20]) thus affecting conversion efficiency. In the present work, oil water content (290-430 mg/kg) was below the maximum value allowed by biodiesel standard EN 14214. On the other hand, statistical analysis showed significant differences concerning AV between FDR and UCC (underlined figures, above 95%), as depicted in Table 1. Due to the high acid value, SFW oil samples from FDR required acid esterification pretreatment before transesterification [8].

4.1.3.2 Chemical composition of FAME

Figure 2 shows the profile of FAME from SFW oil. The predominant fatty acid was oleic acid, C 18:1 (IR: 41.06%, FDR: 50%, UCC: 36.39%, w/w), followed by linoleic acid, C18:2 (IR: 25.71 %, FDR: 21.37 %, UCC: 38.63 %, w/w) and palmitic acid, C16:0 (IR: 18.76 %, FDR: 16.72 %, UCC: 12.38 %, w/w). All SFW oils showed a high content of unsaturated fatty acid methyl esters (IR: 70%, FDR: 74%, UCC: 81%), while the content of saturated fatty acids was lower in comparison to unsaturated ones. However, their presence was significantly high, as result of the heterogeneous matrix of food waste, where lipids and fats from meat, milk, fruit, vegetable, fish, etc. may be found. ANOVA shows differences (Table 2, underlined figures) in the distribution of fatty acid methyl esters. Biodiesel yield (Table 2) is in a range between 80% and 90%, below the minimum threshold required by the European biodiesel standard UNE EN 14214.

UCC samples present significant statistical differences in terms of fatty acid composition (95% significance) with respect to other restaurants. In this sense, UCC samples have similar amount of oleic and linoleic acid methyl esters, whereas FDR samples depict higher content of oleic acid methyl ester (50% w/w) than linoleic acid methyl esters. IR shows higher content of saturated methyl esters, i.e. palmitic and myristic acid methyl esters, while the presence of unsaturated ones is closer among restaurants (Figure 2).

The high content of glycerides and low FAME yield are consistent with an incomplete transesterification reaction (Table 2). In particular, high triglyceride content may cause problems to the engine during storage, besides glycerol separation and increase of aldehydes emissions. However, according to Černoch et al. [21], the impact of triglycerides is not significant below 10% w/w, probably meaning that the standard value for triglyceride content is highly conservative. In this work, the highest value (4.28%) was depicted by IR.

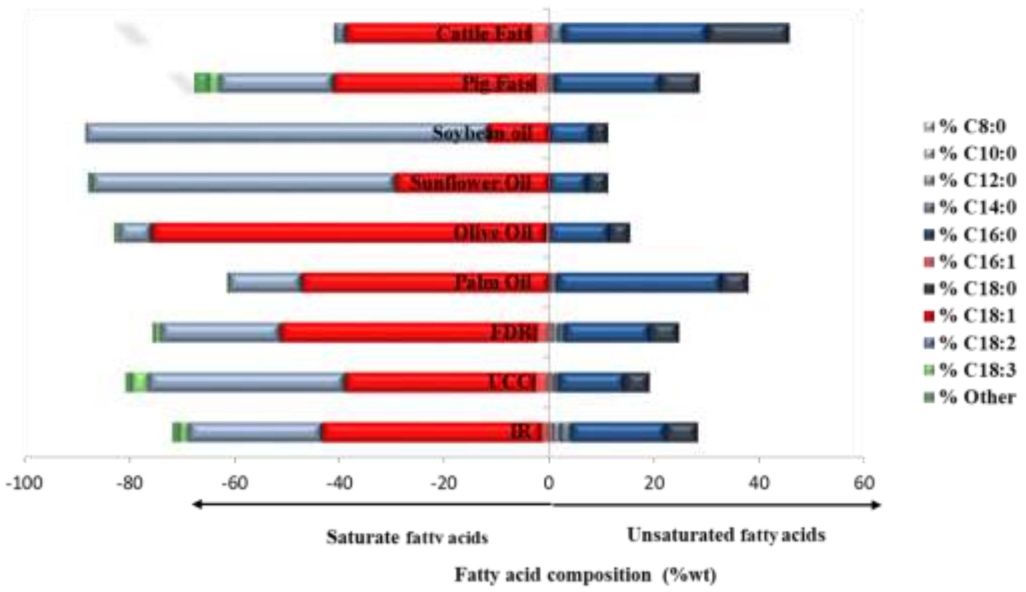

Figure 4.1-2 Fatty acid methyl ester profile from restaurant[2]

To gain knowledge about the implications of the chemical structure and differences in composition over biodiesel properties, two properties have been calculated, namely length of chain (LC) and unsaturation degree (UD) (Table 2). According to the literature, both parameters show a significant impact on biodiesel properties, optimal values being 1.16 for UD and 17.0 carbon atoms for LC [22].

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[2] Italian restaurant (IR), fine dining restaurant (FDR) and University campus cafeteria (UCC) and their comparison with that of most common vegetable oils and animal fats. PCA matrix data was prepared by authors with own data and data from [12]

Table 4.1-2. FAME composition, statistical analysis, chain length (LC) and unsaturation degree (UD)[3].

Composition	Standard test and threshold	Restaurant			ANOVA	
		IR	**FDR**	**UCC**	**P-value**	**LSD test**
FAME (% w/w)	UNE EN 14214 Min: 96.50	86.89 ± 2.88	83.99 ±1.53	87.92 ±0.64	0.339	
C12:0 *(% w/w)*	UNE EN 14103	1.22 ± 0.33	1.25 ± 0.28	0.27 ± 0.18	0.026	UCC vs FDR
C14:0 *(% w/w)*		2.23 ± 0.42	1.09 ± 0.35	0.54 ± 0.13	0.04	UCC vs FDR-IR
C16:0 *(% w/w)*		18.76 ± 1.29	16.72 ± 0.81	12.38 ± 1.60	0.011	UCC vs FDR-IR
C18:0 *(% w/w)*		6.74 ± 0.75	5.36 ± 0.36	4.13 ± 0.55	0.029	UCC vs IR
C18:1 *(% w/w)*		41.57 ± 2.75	49.99 ± 2.13	36.39 ± 2.39	0.009	UCC vs FDR
C18:2 *(% w/w)*		24.85 ± 3.28	21.37 ± 2.94	38.63 ± 2.39	0.01	UCC vs FDR-IR
FG (% w/w)	UNE EN 14105 Max: 0.02	0.98 ± 0.05	0.97 ±0.04	1.26 ±0.07	0,67	--
MG (% w/w)	UNE EN 14105 Max: 0.02	0.89 ± 0.18	0.83 ± 0.09	0.86 ± 0.09	0,946	--
DG (% w/w)	UNE EN 14105 Max: 0.2	0.28 ± 0.15	0.21 ± 0.08	0.11 ± 0.04	0,503	--
TG (% w/w)	UNE EN 14105 Max: 0.2	4.28 ± 1.01	3.88 ± 1.74	2.63 ± 0.86	0,633	--
CR *(% w/w)*	UNE EN 1405 Max: 0.30	0.04± 0.02	0.05±0.02	0.02±0.00	0.295	--
UD[1]		0.98 ± 0.05	0.97 ±0.04	1.26 ±0.07	0.03	UCC vs FDR-IR
LC[2]		17.12 ± 0.21	17.40 ±0.72	17.40 ±0.77	0.2	--

[1] UD= (1%$_{MU}$ +2%$_{DU}$ + 3%$_{TU}$)/100, where %$_{MU}$ is the percentage in weight of monounsaturated methyl ester, %$_{DU}$ is the percentage in weight of diunsaturated methyl ester and %$_{TU}$ is the percentage in weight of triunsaturated; [2] LC =$\sum(nC_n c_n)$, where nC_n is the number of carbon atoms for each fatty acid and c_n is the percentage in weight of each methyl ester containing this fatty acid [12]; CR: carbon residue; FAME: fatty acid methyl ester

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[3] IR: Italian restaurant, FDR: fine dining restaurant, UCC: University campus cafeteri

According to Table 2, UD shows different statistic trends (underlined figures) between restaurants (P= 0.03). It is important to notice that IR UD (0.98) and FDR UD (0.97) show similar values to that of FAME from vegetable oils, i.e. palm oil (0.61) and olive pomace oil (UD 0.98) [12]. On the other hand, UCC UD (1.26) is close to that of sunflower oil (1.51) or maize oil (1.39) [12]. UCC samples depict the highest value of UD due to the presence of unsaturated fatty acids, being more heterogeneous among intra-samples than other restaurants; this high UD value is due to a significant presence of double or triple bonds, which could create parallel reactions, such as auto-oxidation and can affect biodiesel oxidation stability.

As shown in Table 2, oil LC from the three restaurants exhibits a similar value, also comparable to FAME from vegetable oils (sunflower oil biodiesel LC: 17.84; palm oil biodiesel LC: 17.06; olive pomace oil biodiesel: 17.81) [12]. In this sense, IR oil depicts the lowest value (average value of LC being 17.12) compared to other restaurants (UCC: 17.40; FDR: 17.40). This is due to the higher presence of short fatty acid chains (C6:0 to C14:0) besides palmitic acid methyl esters. High content of short fatty acids may enhance some biodiesel properties, i.e. cold behavior performance, □ and oxidation stability [12, 23]. However, no significant statistical differences may be appreciated.

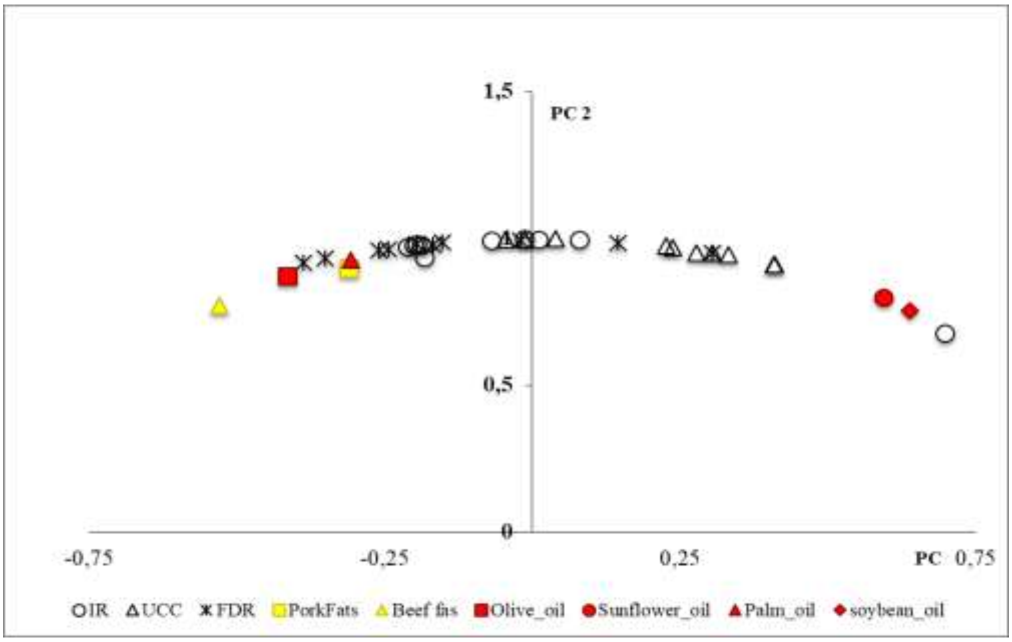

Figure 4.1-3. Correlation matrix between two principal component analysis (PCA) for restaurant SFW oil FAME, vegetable oil FAME and animal fat FAME.

PCA results indicate whether there are similarities between fatty acid profiles, including vegetable oil FAME and animal fat FAME (Figure 3). Considering FAME composition, statistical differences between samples may be found. It can be seen that samples from IR and FDR show strong similarities with palm and olive oil FAME, while oils extracted from UCC SFW are closer to sunflower and soybean oil FAME. With respect to animal fats, SFW samples have a stronger correlation with pork fats. PCA can be explained by two principal components, where the first component (x-*axis*; related to the ratio between oleic and linoleic fatty acids) explains most of the statistical differences (84.6% of variance). In fact, most samples from UCC are grouped around sunflower and soybean oil FAME, which are characterized by a high content of linoleic acid. However, IR and FDR samples are grouped close to olive and palm oil FAME (palmitic acid has also a positive correlation considering the first component) and meat fat fatty acids, with a significant presence of oleic and palmitic acids. The differences considering UD values (Table 2) may also be explained with the first component, due to this ratio. On the other hand, the second component (y-*axis*) provides information about the length of chain of FAME. Analysis indicates a low variability (9.25% of variance) with a weak correlation between palmitic (C16) and stearic (C18) fatty acids. The study of these components sheds light over LC values (Table 2), which are influenced by the content of palmitic acid.

4.1.3.3 Biodiesel properties

SFW oil biodiesel properties were analyzed (Table 3). Results indicate that SFW provides a feedstock that may be used to produce FAME. In fact, ϑ, CR, FP, humidity, linolenic methyl ester content, ν and CN meet the European standard EN 14214. However, as previously mentioned (Table 2), biodiesel yield is in a range between 80% and 90%, below the minimum threshold required by the European biodiesel standard EN 14214. Moreover, oxidation stability and, in some cases, glyceride content do not meet EN 14214. Therefore, to improve these properties, pre- and post- treatments should be studied.

In terms of storage, oxidation stability values are below the regulations (EN 14124). This fact can be related to the thermochemical processes that take place during cooking and manipulation of food. In this regard, antioxidants, such as N, N'-diphenyl-1,4-phenylenediamine and N-phenyl-1,4-phenylenediamine can increase this property, as reported by Rashed et al. [24].

Biodiesel CR is an important property, which gives an approximate measure of the carbon-settling tendency. The presence of glycerides, free fatty acids, polyunsaturated esters and polymers (in case of used frying oils) can increase CR [25]. In this case, all samples were several times below the standard threshold. Best results were provided by UCC (0.02%), followed by IR (0.04%).

As previously explained, biodiesel CN was calculated using a prediction model based on UD and LC values from Table 2 [12, 26]. This property provides information about the ignition delay time (ID) upon injection into the combustion chamber. High values indicate less ID, while elevated CN values are related to low values of UD and high values of LC. Best result was provided by FDR (58.44), while UCC showed the lowest value (53.97) which is in agreement with its unsaturated composition. On the other hand, high values of CN could help reducing NO_x emissions [26].

Table 4.1-3. Properties of biodiesel from restaurant solid organic waste oil according to European biodiesel standard 14214[4].

QUALITY PARAMETERS			**RESTAURANT**		
PROPERTY	**Unit**	**Standard test and threshold**	IR	FDR	CUC
ν at 40 ºC	(mm^2/s)	EN ISO 3104 3.5-5.0[a]	3.91 ± 0.01	4.84 ± 0.11	4.44 ± 0.02
LCV	(kJ/kg)	ASTMD240 Min: 35000	39454.00 ± 103.50	39572.00 ± 61.29	39496.67 ± 72.59
CFFP	(ºC)	EN 116 14214*	-2.67 ± 0.33	-5.67 ± 0.33	-9.00 ± 0.00
ϑ	(mg/kg)	EN ISO 12937 Max: 500[a]	213.59 ± 14.32	291.82 ± 13.52	137.63 ± 16.39
ρ at 15ºC	(kg/ml)	EN ISO 3675 860-900[a]	875.67 ± 1.45	887.00 ± 1.15	874.33 ± 1.45
FP	(ºC)	EN 14105 Min: 122ºC	81.67 ± 2.03	164.33 ± 3.48	91.33 ± 1.20
CR	(% w/w)	UNE EN 14105 MAX:0.30	0.04±0.02	0.05±0.02	0.02±0.00
OXS	(h)	UNE EN 14105 Min: 6	3.54 ±0.74	2.10±0.51	2.30±0.29
CN		UNE EN 14105 Min: 50	57.76±1.07	58.44±0.64	53.97

NOTES: ν: kinematic viscosity; LCV: low calorific value; CFFP: cold filter plugging point; ρ: density; FP: flash point; CR: carbon residue; OXS: oxidation stability; CN: cetane number; ϑ: water content. Limit established by EU standard EN 14214 (a). *no specific values, depending on the climate**.**

Biodiesel water content is below the standard threshold (500 mg/kg, according to EN 14214), thus avoiding microbial growth (promoting fuel filter plugging) and corrosion of both storage and engine fuel systems [27]. Also, kinematic viscosity is in agreement with European standard, showing values between 3.5 and 5 mm^2/s. FAME obtained from FDR depicts the highest kinematic viscosity value (4.84 mm^2/s), followed by UCC (4.44

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[4] IR: Italian restaurant, FDR: fine dining restaurant, UCC: University campus cafeteria.

mm^2/s) and IR (3.91 mm^2/s). Kinematic viscosity affects injection and combustion efficiency [12]. This property increases with the length of chain or glyceride content. In the present work, high values of kinematic viscosity may be due to the presence of glycerides, besides the high content of trans-unsaturated fatty acids (when the oil undergoes processes such as frying, cis bonds are transformed into trans ones) [28].

In terms of cold behavior properties, CFPP indicates whether the fuel can crystallize and clog the filter, causing problems in compression-ignition engines. Although, there are no specific limits for CFPP in EN 14214, EN 590 for diesel fuel regulates two weather groups. In this sense, CFPP limits for Spain are set on 0ºC for summer season and -10ºC for winter. CFPP results from this work are shown in Table 3. It may be seen that IR FAME exhibits the worst value (-2.33ºC) due to its high content in saturated fatty acids, i.e. palmitic and stearic methyl esters. The higher the value of UD, the better the cold behavior properties. This is in agreement with results, as UCC biodiesel presents a value of UD of 1.23, being CFPP the lowest value [29].

Results concerning FP, which reflect the risk of fire hazard, are shown in Table 3. It may be seen that only FDR FAME FP meets the standard (163.33ºC), while FAME from IR and UCC present FP values below the limit (81.67ºC and 91.33 ºC, respectively). FAME from SFW is less dangerous than petrodiesel (FP values between 38ºC until 55ºC) or diesel fuel from pyrolysis (45.3ºC). The number of carbons is the most influential factor affecting FP [12]. In this work, all samples show similar LC values (Table 2, IR: 17.12, FDR and UCC: 17:40) also similar to those of vegetable oil and animal fat methyl esters (sunflower oil methyl ester: 17.54, palm oil methyl ester: 17.12, line oil methyl ester: 16.11) [22]. FP value from UCC samples may be related with the boiling points of methyl esters (BP) [30].

HCV is another important parameter, together with CN, that reports information about energy in fuel that may be transferred during combustion. FAME (39-41 MJ/kg) exhibits lower values than diesel fuel (43 MJ/kg), but higher than coal (32-37 MJ/kg) [12, 31]. The higher the number of carbon, hydrogen and oxygen atoms, the higher the value of HCV. Therefore, for biodiesel production, oils with elevated saturation degree (high C, H_2 and O_2 content) are preferred to higher unsaturated ones [12]. For heating purposes, a minimum LCV value of 35 MJ/kg should be met. In this work, LCV was calculated from HCV excluding the heat of vaporization of the water. As can be observed from Table

3, no significant statistical differences were found between restaurants, being LCV in the range of 35 MJ/kg.

Conclusion

In this study, the suitability of oil from solid food waste (SFW) as raw material for FAME production has been analyzed. Samples of oils extracted from SFW from different restaurants showed some significant differences in fatty acid composition, but similar to that of vegetable oils. In spite of these differences, physical and chemical properties were similar between oils, with the exception of acid value. Consequently, it seems possible to work with mixtures of oils extracted from SFW from different restaurants to produce biodiesel. In this case, as these oils show a strong tendency to present a high content of free fatty acids, a preliminary acid value analysis and further acid catalyzed-esterification pre-treatment are needed. Several chemical and physical properties of SFW oil biodiesel were analyzed. It was observed that only oxidation stability, FAME yield and glyceride content did not fulfil European standard EN 14214. For this reason, to improve biodiesel quality, reaction optimization, blending with diesel fuel or the use of additives are highly recommended. As a general conclusion, oil from SFW may be recommended to produce biodiesel, blended with diesel fuel or with additives, thus helping to reduce costs in restaurants, recycling residues and mitigating exhaust emissions produced from the combustion of fossil fuels.

4.2 OPTIMIZATION OF SOLID FOOD WASTE OIL BIODIESEL BY ULTRASOUND-ASSISTED TRANSESTERIFICATION

Abstract

Substitution of fossil diesel fuel by biodiesel is a realistic alternative due to its compatibility with commercial diesel engines. However, detractors consider that traditional raw materials used for biodiesel production may compete with human feeding (edible vegetables oils), land and water. So, new raw materials are needed. Annually, around 1.3 billion ton of food are discarded or wasted. The main goal of this work is biodiesel production optimization (with and without auxiliary energy assistance), and further characterization, by using the lipid fraction included in solid food waste, collected from local restaurants. Solid food waste oil (SFWO) characterization showed a fatty acid profile rich in oleic (C18:1), palmitic (C16:0) and linolenic (C18:2) acids, a remarkable fatty acid composition suitable for biodiesel production. Due to high free fatty acid content, acid esterification pre-treatment was conducted, followed by a basic transesterification optimization, by both conventional and ultrasound (US) assisted reactions. Response surface methodology was selected to perform the experimental design; optimal conditions for conventional transesterification were achieved at 6.08:1 methanol-to-oil molar ratio, 1.28% w/w catalyst and 52.5ºC reaction temperature, providing 93.23% w/w fatty acid methyl esters (FAME) conversion. Biodiesel quality was also analyzed with excellent results considering cold properties (cold filter plugging point below -3ºC), although oxidation stability did not fulfill European standard limit. US-assisted reaction was also compared with conventional transesterification in terms of energy consumption and reaction time, providing significant savings in both energy and reaction time (40 min reduction). As a conclusion, US-assisted SFWO biodiesel meets most European standard limits, following circular economy. To extend its use, blends with diesel fuel and antioxidant addition are recommended, besides long-term engine tests.

4.2.1 Introduction

Over the next 50 years, human society must find solutions to multiple challenges due to exponential population growth. One of the most important issues to be faced derives from the use of fossil fuels. Oil price increase (due to fluctuations in the markets), environmental and health deterioration, besides geopolitical instability, are taking special relevance and require short-term resolution.

For the reasons above, a growing interest in searching for new biofuels as a replacement of fossil fuels that are compatible with internal combustion engines has been promoted. Biofuels are becoming a new alternative to reduce both dependency on fossil fuels and greenhouse gas emissions. Biofuel sector produces 133 billion liters per year and employs a total of 1 678 000 people [32]. In this industry, bioethanol and biodiesel are the world´s most abundant biofuels. Most biodiesel production is used in the transport sector. Considering last 15 years, its production has grown by 6.5%. Nevertheless, despite the tendency of growth in the previous years, biodiesel production in 2015 was 30.1 billion litres, which represents one per cent of decrease in growth with respect to 2014. It may be due to an increase in raw material prices and restrictive policies in the use of edible crops [32]. Furthermore, 95% of biodiesel feedstocks come from edible crops such as palm, soybean and rapeseed oils [33]. According to the literature, intensive soil use could negatively affect the food supply chain and environmental equilibrium [34]. This fact has led to search for alternatives to these edible crops, being the use of non-edible ones among the first evaluated options. In this way, species like *Jatropha curcas*, *Camelina sativa* or *Sinapis alba* have been tested with interesting results.

Given the controversy regarding biofuel production, both industry sector and scientific community are evaluating new feedstocks. In this way, the use of algae, microalgae or other microorganisms may provide a long-term fuel alternative. Nowadays, high costs and low process efficiency are the main objections to widen their acceptance. In this context, food waste represents an interesting unexplored alternative. The use of food waste as raw material may help reducing biodiesel final costs, thus placing this residue among the most competitive alternatives to produce biodiesel *versus* fossil fuel.

According to Food and Agriculture Organization of the United Nations (FAO) report [35], close to one third of food production (1.3 billion ton) is annually discarded or wasted. The habits of consumers, especially in developed countries, generate daily significant food waste. Besides, waste management represents a very expensive environmental problem. This residue usually presents highly heterogeneous composition [36]. In terms of lipid content, food waste includes a liquid phase (oil), besides a solid fraction (fat), at room temperature. Solid fraction may be composed of wax and grease residues. Grease originates from used oils and fats, which may exhibit high free fatty acid (FFA) content [37]. Considering food waste recycling, lipid fraction plays a significant role. In this sense, during anaerobic digestion (AD) it may depict a negative effect as it is a limiting factor in methane production; to improve AD economic viability, lipid fraction must be removed from solid food waste (SFW), under a costly and time-consuming process. Biodiesel production is an attractive option to valorize this fraction of SFW. In this sense, SFW lipid fraction may be as interesting as other oily residues from kitchens and food industries, i.e. waste oil, grease, beef/lamb tallow and fish oil [38]. Although they present disadvantages, i.e. high water content and FFA value [9], appropriate processing may overcome these problems [39].

In this scenario, new technologies may be essential tools to strengthen last-generation biofuel introduction in the market. As an example, organic chemistry frequently needs to produce emulsions in heterogeneous liquid-liquid systems. This is the case of transesterification, that is the chemical reaction leading to biodiesel synthesis. Reaction occurs between two phases that are almost immiscible; on one side, triglycerides that are present in vegetable oils and animal fats and, on the other side, short chain alcohol (methanol or ethanol) in the presence of acid or basic catalyst. Transesterification includes heating and stirring reaction mixture to put in contact, as much as possible, both almost immiscible phases, to achieve high conversions in the shortest period of time. This process is, in most cases, slow and inefficient considering energy consumption. Assisting reaction with auxiliary energies, i.e. ultrasound (US), microwave and radiofrequencies, biodiesel synthesis requires lower energy consumption, while providing environmental benefits [40]. Sáez-Bastante *et al.* [41] demonstrated that US-assisted transesterification reduced reaction inputs (methanol-to-oil molar, energy and reaction time). Therefore, energy requirements are lower than those of conventional transesterification.

Physically, US is an acoustic wave with a frequency above the limit of human earing (approximately, 20 kHz), generated by local variation, in density or pressure, into a continuous medium. Transmission is possible because each molecule transmits the vibration to its adjoining molecule, causing a chain movement and returning approximately to its original position [41]. Assisting reaction medium with US provides a physical phenomenon, known as cavitation, that is responsible for physical and chemical mechanisms that accelerate reaction. Cavitation consists in the formation and further growth of gaseous microbubbles, which implode because external pressure is higher than internal one. This leads to high local temperatures and subsequent emulsifications in short periods of time [41].This phenomenon is especially useful in biodiesel synthesis to increase reaction rates and reduce alcohol-to-oil molar ratios, because it allows an intimate contact between interphase area oil-alcohol [42]. To the best of our knowledge, there is no research concerning the use of the lipid fraction from SFW to produce biodiesel via US-assisted transesterification. The strength of this claim is based on the need to recycle food waste, on one hand, and the production of last-generation biofuels under an efficient method, on the other.

The present work aims to study last-generation biodiesel production using extracted lipids, included in SFW from different restaurants. The work is assisted by response surface modeling. Conventional method combining both agitation and heating is compared to US-assisted transesterification (using a probe as ultrasonic device), in terms of both biodiesel yield and energy consumption. Finally, to evaluate the quality of produced last-generation biodiesel, physical and chemical properties are analyzed under European biodiesel regulation EN 14214

4.2.2 Materials and methods

Figure 1 shows the different steps carried out in this study, ranging from oil recovery to biofuel synthesis, including pretreatment and characterization.

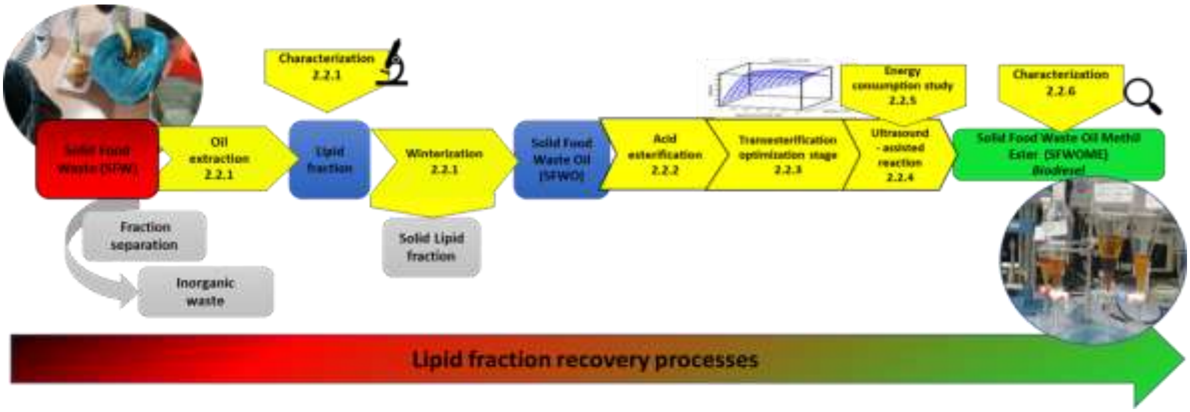

Figure 4.2-1. Lipid fraction recovery processing from solid food waste.

4.2.2.1 Raw materials

Solid food waste (SFW) samples were collected from four local restaurants based in Cordoba (Spain). They were stored during several weeks, until a representative pull of SFW samples, comprising a variety of food processing establishments was guaranteed. Sample collection, pre-treatment and oil extraction were described in a previous work by Carmona-Cabello *et al.* [36].

4.2.2.2 Reagents

Pre-treatment (acid esterification) needed methanol ACS-ISO and sulfuric acid (96% purity, Pa-ISO) from Panreac (Barcelona, Spain). For transesterification, methanol (ACS-ISO) and KOH pellets (85% purity), all supplied from Panreac Química (Barcelona, Spain), were used.

Reagents needed to quantify fatty acid methyl esters (FAME) and glyceride concentration were methylheptadecanoate (internal standard), 1,2,4-butanetriol, 1,2,3-tricaprin solution (8000µg/ml in pyridine) and N-methyl-N-trimethylsilyl trifluoroacetamide (for derivatization) all from Sigma-Aldrich (St. Luis, MO, US). Also, n-heptane, from Panreac Química (Barcelona, Spain) was used as solvent for FAME standard solution. Acid value determination required 2-propanol, toluene, phenolphthalein, ethanol, benzoic acid and potassium hydroxide, all from Panreac Química (Barcelona, Spain).

4.2.2.3 Oil extraction and characterization

According to Carmona-Cabello *et al.* [36], lipid fraction was separated from SFW by Soxhlet method. Then, to reduce the content of saturated fatty acids (solid lipid fraction), winterization was carried out under centrifugation, during 10 min, at 0°C and 2000 rpm. Fatty acid composition, FFA content, kinematic viscosity and water content of oil fraction were characterized following standards and methods described by Carmona-Cabello *et al.* [36].

4.2.2.4 Esterification pre-treatment

Due to high FFA, prior to transesterification, a esterification step was needed. Reaction was performed heating at 65ºC a solution of 1% w/w H_2SO_4 and 8:1 methanol/oil molar ratio, mixed with oil from the liquid lipid fraction, during 120 min. Sampling was carried out at 1, 2, 5, 15, 45 and 120 min. To remove impurities, samples were centrifuged. To find the optimum reaction time, acid value was analyzed following EN ISO 660 standard.

4.2.2.5 Conventional transesterification optimization by response surface methodology

Response surface methodology (RSM) was used to optimize FAME production under alkali-catalyzed transesterification. Box-Behnken design with 15 runs and three centre points was used. Methanol-to-oil molar ratio and catalyst concentration were used as chemical reaction input factors; temperature was considered as chemical reaction input factor, whereas FAME yield was set as response variable. Experimental design and data treatment were performed with the help of Statgraphics Centurion XVI software (Statpoint Technologies, Warrenton, Virginia, US).

Subsequently, conventional transesterification was carried out using optimal reaction conditions provided by RSM. Kinetic study was carried out by sampling at 30 s, 1, 2, 5, 10, 20, 40 and 60 min. Temperature was controlled using a heater-stirrer Ovan MBG05E (providing a maximum power of 500 W) purchased from Espier Group (Barcelona, Spain).

4.2.2.6 Ultrasound-assisted transesterification

Once reaction parameters were optimized, transesterification was carried out using US as auxiliary energy to reduce power consumption. This study allowed reaction time optimization. Sample ultrasonication was carried out using a Sonicator probe Q700 model, made of titanium alloy (0.5 inch of diameter) supplied by QSonica LLC (Newtown, US). The frequency provided by the probe was 20 kHz, allowing tuning both amplitude and duty cycle. In addition, the probe provides energy consumption data. 100% duty cycle and 50% amplitude were selected.

2.2.5 Energy consumption study

In conventional transesterification, energy consumption studies were carried out using two Fluke power analyzers (Everett, Washington, US) which worked at different voltage range. Fluke three-phase power quality analyzer model 435 reaches maximum input voltage of 1000 V rms (root-mean squared voltage), works in a nominal voltage range of 50-500 V, maximum peak voltage of 6 kV, nominal input current of 0.1-3000 A rms and nominal frequency range of 40-70 Hz. Fluke power quality analyzer model 43B shows maximum input voltage of 1250 V rms, nominal voltage range of 50-500 V, maximum peak voltage of 6 kV, nominal input current of 0.5-40 A rms and nominal frequency range of 40-70 Hz.

Considering US-assisted transesterification, power measurements were carried out by connecting both ultrasonic horn and heater-stirrer Fluke 435 analyzer (Everett, Washington, US). To control voltage and frequency from electric current outlet, Fluke 43B analyzer was connected to electric current. Working voltage and frequency were 229.1 V and 50 Hz, respectively. Energy consumption was evaluated using a parameter introduced by authors, named *energy use index* (EUI). This parameter provides information about energy efficiency, being the ratio between released energy per mass unit after combustion (low calorific value, LCV) and supplied energy per mass unit, during its synthesis [43]. It was calculated according to equation 1.

$$EUI = \frac{Low\ calorific\ value\ (\frac{J}{g})}{Amount\ of\ energy\ per\ mass\ unit\ required\ for\ its\ synthesis(\frac{J}{g})} \quad (1)$$

4.2.2.7 Biodiesel characterization

According to EN 14103 standard, FAME yield was measured using a Perkin Elmer GC model Clarus 500 (Waltham, Massachusetts, US) coupled to a flame ionization detector and comprising a 30 m × 0.25 mm Elite 5-ms (0.25 µm particle diameter) capillary column. Mono- (MG), di- (DG) and triglycerides (TG) and glycerol determination was carried out following EN 14105 standard, using SGE BPX5 capillary column (12 m length, 0.32 inner diameter and 0.25 ml film).

Oxidation stability was analyzed in a professional biodiesel Rancimat, from Metrohm (Herisau, Switzerland) following EN 14112 standard. Carbon residue (CR) was determined according to EN 10370 standard using micro carbon residue tester (Alcor MCRT-160 by PAC, Houston, US). High calorific value (HCV) was analyzed using an IKA bomb calorimeter C200 (Staufen, Germany) and following ASTM D240 standard. Density (ρ) was measured following EN ISO 3675 protocol. Acid value (AV) was analyzed following EN14104 standard. Kinematic viscosity (ν) was determined using a glass capillary-type viscometer (Cannon-Fenske, size 150) following EN ISO 3104 standard. Water content was measured using a Karl Fischer titrator, model DL32 Mettler Toledo (Columbus, US) following EN ISO 12937 standard. Cetane number (CN) was calculated according to Pinzi *et al*. [44]. Flash point (FP) was analyzed by Seta Flash series 3 plus, from Instrumentation Analitica S.A. (Madrid, Spain) according to EN ISO 3679. Cold filter plugging point (CFPP) was measured following EN 116 standard, by means of HCO 842 (Herzog by Pac) equipment. Metal content was measured by inductively coupled plasma mass spectrometry (ICP-MS) NexION 350X, from PerkinElmer (Waltham, MA, US) considering threshold from standard EN 14538

4.2.3 . Results and discussion

4.2.3.1 Fatty acid composition

Table 4.2-1. Characterization of solid food waste oil (SFWO)

FATTY ACID	COMPOSITION (% w/w)
Caprylic acid (C8:0)	1.7 ± 0.1
Capric acid (C10:0)	1.9 ± 0.1
Lauric (C12:0)	1.72 ± 0.07
Myristic (C14:0)	0.15 ± 0.12
Palmitic (C16:0)	16.60 ± 0.65
Palmitoleic (C16:1)	1.69 ± 0.07
Stearic acid (C18:0)	6.10 ± 0.31
Oleic (C18:1)	46.00 ± 1.89
Linoleic (C18:2)	23.00 ± 1.05
Linolenic (C18:3)	1.10 ± 0.10
HYDROCARBON CHAIN PROPERTIES	
LC[1]	17.15
UD[2]	0.98
PHYSICAL AND CHEMICAL PROPERTIES	
Acid value, AV (mg KOH/g)	7.53 ± 0.06
Water content (ppm)	521 ± 62
Kinematic viscosity at 40ºC, ν (mm^2/s)	24.60 ± 0.67

[1] Length of chain, LC = Σ(*nCn*)/100, where *n* is the number of carbon atoms of each fatty acid and *Cn* is the weight percentage of each fatty acid methyl ester; [2] Total unsaturation degree, UD = (1 %*MU* + 2 %*DU* + 3 %*TU*)/100, where *MU* is monounsaturated methyl esters (% w/w), %DU is diunsaturated methyl esters (% w/w) and %TU is triunsaturated methyl esters (%, w/w)

The lipid fraction from SFW is a complex mixture of fatty acids from different origin, i.e. meat and fish fat, vegetable oil used during cooking, etc. It also suffers diverse heating processes (frying, cooking, grilling), chemical degradation (Millard and hydrolytic reactions) and microbial attack. Once the solid fraction was removed, fatty acid composition and some properties of the liquid lipid fraction were determined (Table 1).

Principal component analysis (PCA) was used to compare this fatty acid profile with that from a wide variety of popular oils/fats (including edible and no-edible oils, animal fats, grease and waste cooking oil) used for biodiesel production [45, 46]. In Figure 2, statistical results from PCA are presented. First component (*x*-axis) distributes samples considering oleic (C18:1) and palmitic fatty acid (C16:0) concentration; second component (*y*-axis) helps to explain its behavior according to linoleic acid proportion

(C18:2). PCA analysis allows to establish three different clusters: cluster number one shows oils where linoleic acid (C18:2) is the main component, i.e. poppyseed or soybean oils (edible oils). This cluster is mainly composed by oils with high unsaturation degree (UD). Cluster number two is composed by oils with a high proportion of mono- (C18:1) with respect to di-unsaturated fatty acid (C18:2). This composition was described as ideal for biodiesel production [44]. Finally, cluster number three shows oils with a ratio around 2:1 of C18:1-C16:0. This cluster includes oils with shorter chain length fatty acid. It has been found that this composition is preferred for properties like viscosity or low temperature flow behavior [47]. The rest of samples depicts a wide variability of fatty acid profiles. SFWO is included between clusters no. two and three, as its profile is 1:2:1 (C16:0-C18:1-C18:2). Oleic fatty acid shows the highest proportion (45.8%, w/w) followed by linolenic fatty acid (23%, w/w) and palmitic fatty acid (16%, w/w). SFWO stands out compared to other oils because of the balanced ratio between palmitic and linolenic. According to previous studies [23, 44], its profile is optimal to reach biodiesel physical and chemical properties closer to the ideal formula to fuel compression ignition engines.

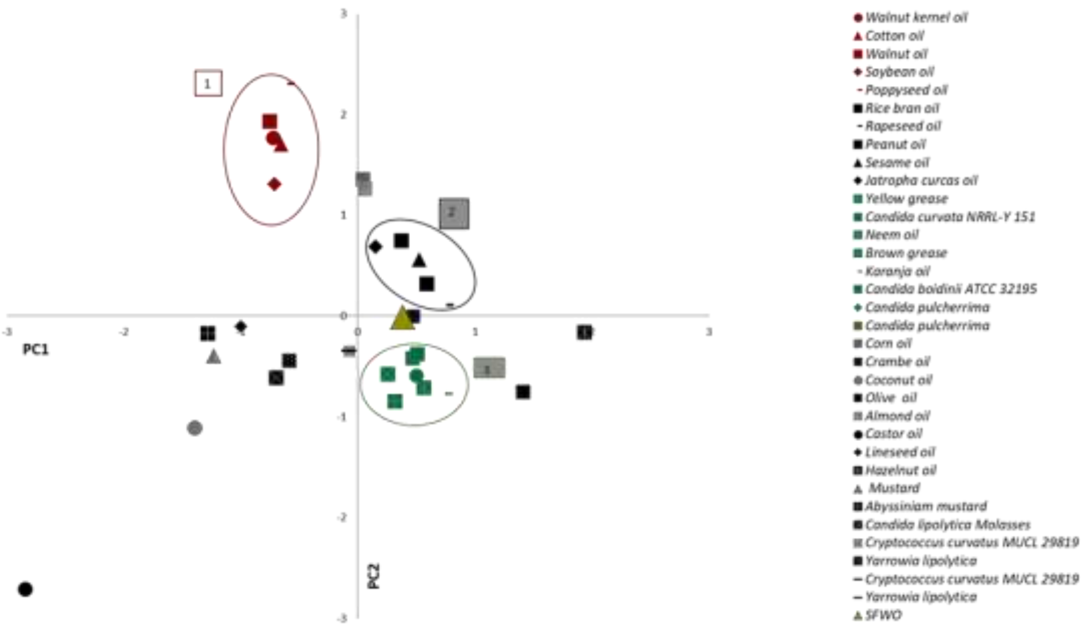

Figure 4.2-2. Principal component analysis. PC1: combination of C16:0 and C18:1; PC2: C18:2

4.2.3.2 Acid esterification

It is critical to control AV oil prior to transesterification. Oils/fats including high FFA content may reduce or inhibit FAME conversion during transesterification, due to soap formation and other side reactions [46]. As shown in Table 1, SFWO presents an AV of 7.35 mg KOH/g, which represents 3.71% w/w FFA. Different authors advise against the use of straight transesterification when oil/fat FFA content is high, recommending previous esterification step to reduce acidity. In Table 2, different approaches to establish the allowed limit of oil/fat FFA for direct transesterification use are summarized. Dorado *et al.* [48] indicated that AV values above 3% w/w lead to saponification reactions, which can affect transesterification. In this case, an acid catalyzed esterification pre-treatment is imperative to esterify FFA, followed by an alkali catalyzed transesterification in a second step. According to this and with the aim of lowering AV, while increasing biodiesel yield, SFWO was subjected to acid-catalyzed esterification pre-treatment.

Table 4.2-2. Recommended FFA (% w/w) to allow the use of direct transesterification.

FFA[1] CONTENT (%, w/w)	**REFERENCE**
< 3	Dorado *et al.* [48]
≤ 2	Ramadhas *et al.* [49] and Sahoo *et al.* [50]
< 1	Ma and Hanna [51], Freedman *et al.* [52] and Tiwari *et al.* [53]

[1]FFA: free fatty acids

Figure 3 shows AV evolution over time during acid esterification. This study may help optimizing reaction time during pre-treatment, thus saving time and costs. In this sense, after five min esterification reaction time, AV was below 1% w/w. According to Table 2, AV value below 1% w/w is low enough to avoid side reactions. Esterification step may be stopped at this point, giving way to basic-catalyzed transesterification.

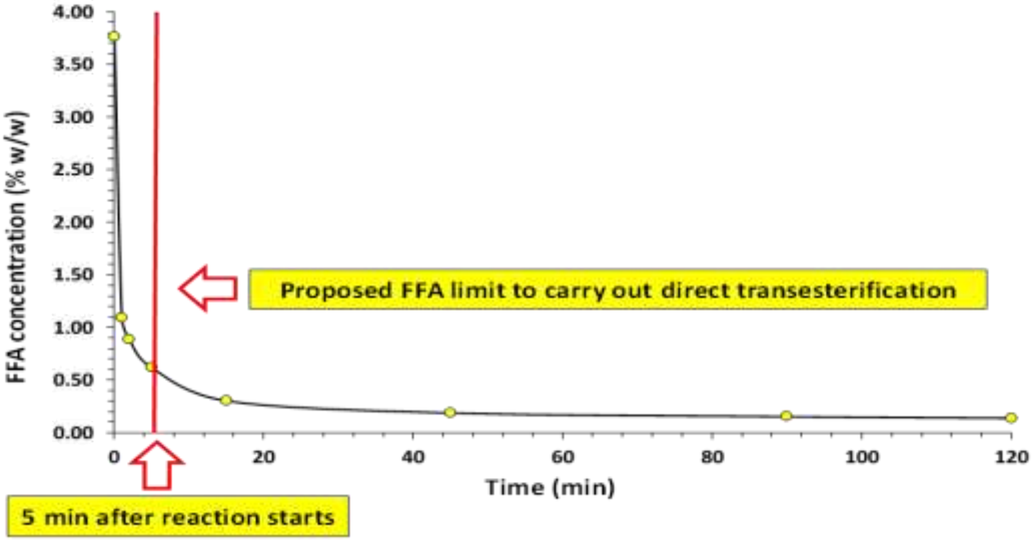

Figure 4.2-3. Evolution of acid value during acid esterification of solid food waste oil. FFA: free fatty acids

4.2.3.3 Conventional transesterification optimization by response surface methodology. Design of experiments

Table 4.2-3 Design of experiments and experimental FAME yield. SFWOME: Solid food waste oil methyl ester.

REACTION PARAMETERS			RESPONSE VARIABLE
Methanol-to-oil molar ratio	Catalyst (%, w/w)	Temperature (°C)	SFWOME yield (%, w/w)
5.5	1.4	52.5	92.19
5.5	1.4	52.5	93.44
6.5	0.8	52.5	93.97
4.5	1.4	65	91.22
5.5	0.8	65	90.23
6.5	1.4	65	90.91
4.5	0.8	52.5	88.88
6.5	1.4	40	91.77
6.5	2	52.5	89.92
5.5	2	65	92.31
5.5	1.4	52.5	93.24
4.5	2	52.5	90.98
5.5	0.8	40	84.74
5.5	2	40	92.22
4.5	1.4	40	90.26

RSM was used to optimize three transesterification reaction parameters from conventional heated-stirred transesterification (methanol-to-oil molar ratio, catalyst concentration and temperature), while reaction time was fixed at one hour, taking into account previous studies of biodiesel synthesis with the same raw material [36]. As previously mentioned, study of the influence of experimental factors on response variable (solid food waste oil methyl ester, SFWOME) was performed by means of a Box-Behnken model.

Table 3 shows experimental FAME conversion after carrying out lab experimentation. As may be seen, yields are usually above 90% w/w, although none of them exceeds the minimum threshold established by European biodiesel standard EN 14214 (96.5%, w/w). Maximum experimental values are below 94% w/w.

Table 4.2-4 Optimal values and statistical parameters from conventional transesterification design of experiment

RESPONSE VARIABLE	
FAME yield (% w/w)	93.23
EXPERIMENTAL FACTORS	
Methanol-to-oil molar ratio	6.08
Catalyst (% w/w)	1.28
Temperature (ºC)	52.5
STATISTICAL PARAMETERS	
R^2 of the model (%)	86.59
Lack of fit (P-value)	0.157
Standard error	0.67
QUALITATIVE EFFECTS OF THE PARAMETERS ON RESPONSE VARIABLE	
A: Methanol-to-oil molar ratio	+
B: Catalyst (% w/w)	+
C: Temperature (ºC)	+

According to results showed in Table 4, predicted optimum yield was 93.23 % w/w. The fitting of model to data was evaluated by means of lack-of-fit P-value, providing a value higher than 0.05 (0.157). Therefore, the model appears to fit observed data at 95% confidence level. It can also be observed that the three reaction parameters have a positive effect on response variable, but it is not significant at 95% confidence level (Table 4).

Figure 4 shows estimated 3D response surface plot of FAME yield at optimal reaction temperature (52.50ºC). Catalyst concentration and methanol-to-oil molar ratio tendencies agree with results showed in Table 4. In this sense, FAME maximum yield is reached in a region with methanol-to-oil molar ratio close to 6:1 and above 1.2% w/w of catalyst concentration. Maximum yield corresponds to a combination of medium concentration of catalyst and high methanol-to-oil molar ratios.

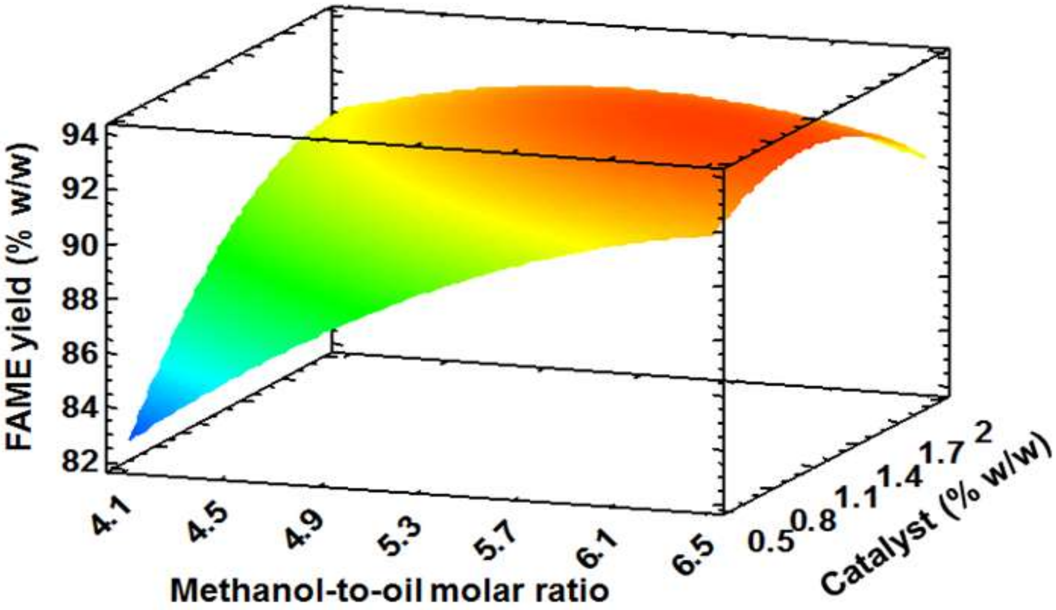

Figure 4.2-4 Response surface for FAME yield at optimal temperature (52.5ºC)

4.2.3.4 Ultrasound-assisted transesterification

To compare conventional transesterification with US-aided transesterification, a comparative study was carried out. During US-assisted reaction, catalyst concentration and methanol-to-oil molar ratio, both optimized for conventional reaction, were used, whereas, room temperature, instead 52.5ºC, was kept during ultrasonicated reaction. Figure 5a shows reaction products evolution *vs.* time. In general terms, transesterification with or without ultrasonication depicted similar FAME conversion. However, conventional transesterification required longer reaction time to reach optimal conversion (approx. 93% w/w), while US-assisted reaction achieved similar conversion in a shorter period of time, saving 40 min of reaction time.

Figure 5b shows glyceride concentration evolution during ultrasonicated reaction. As may be appreciated, during the first five seconds of reaction, a conversion above 80% of glycerides takes place. The lowest glyceride concentration is reached after 500 s of reaction, as FAME yield is close to its maximum value.

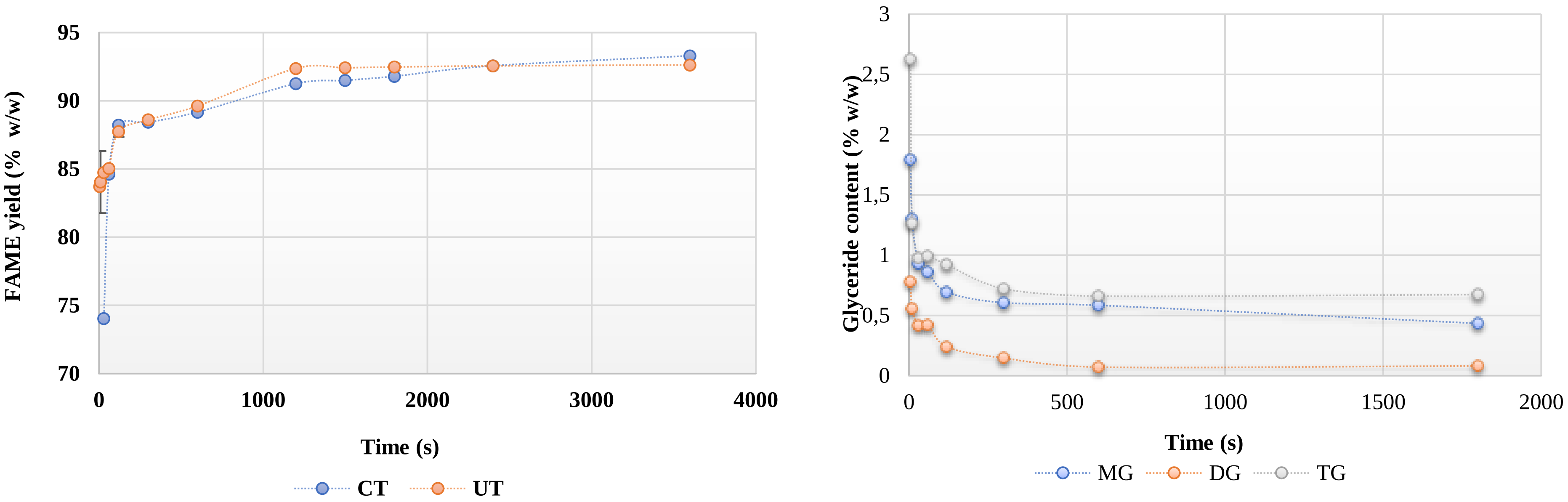

Figure 4.2-5. (a) FAME yield under conventional transesterification (CT) and ultrasonication conditions (UT). (b) Glyceride concentration vs. time during ultrasound assisted transesterification. TG: triglycerides, DG: diglycerides, MG: monoglycerides.

4.2.3.5 Energy consumption study

To compare both types of transesterification, EUI provides useful information. Conventional transesterification includes a heater-stirrer and works under continuous demand of energy (average power demand of 105 W). Meanwhile, US device provides energy consumption under 100% of duty cycle. Considering optimal reaction time (60 min for conventional transesterification and 20 min for ultrasonicated one) EUI was calculated. Although after 20 min reaction time, conventional transesterification provided almost the same value that after 60 min and below that of US-assisted transesterification (Figure 5a), time for maximum yield has been selected. Figure 6 shows results considering that acid pre-treatment was the same for both kind of reactions and, consequently, it presents the same EUI value. In this way, US-assisted transesterification depicts a value of EUI almost five times higher than that of conventional reaction, considering similar FAME yields. This finding involves a significant energy saving during biodiesel synthesis.

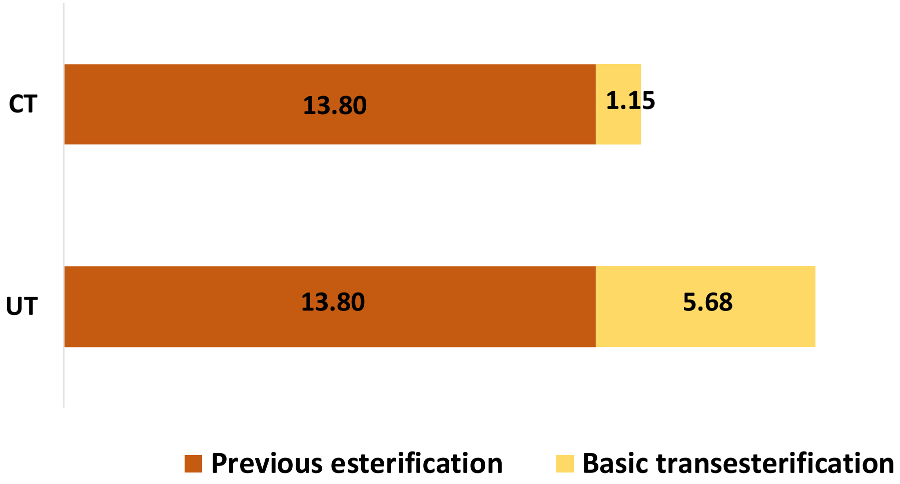

Figure 4.2-6. Comparison of energy use index (EUI) between conventional (CT) and ultrasonicated (UT) transesterification

4.2.3.6 Quality parameters

To be marketed, it is imperative to evaluate biofuel suitability. Considering biodiesel from both transesterifications, Table 5 shows different fuel quality parameters, most of them included in European biodiesel standard EN 14214. It may be appreciated that FAME conversion is above 93% w/w for both processes, but below threshold required by European standard. Yield roof may be attributed to complex combination of different factors. On one hand, the presence of glycerides, slightly above standard limit, shows that reaction was incomplete. On the other hand, although around 5% of FAME composition remains unknown, it contains a certain quantity of phospholipids. According to other researchers, alkali catalysts may be destroyed by the presence of phospholipids, thus being responsible of transesterification inhibition [52, 54]. This may be the main reason why FAME yield and glycerides do not fully meet biodiesel standard. Although the presence of glycerides is correlated with properties such as carbon residue or viscosity, in this case it may be found that both properties are below standard threshold. In fact, high values of kinematic viscosity would affect injection and combustion efficiency [28], while carbon residue gives information about carbon-settling tendency and subsequent compression loss, among other problems [25]. In the present study, SFWOME viscosity, in both cases, is within the accepted range (3-5 mm^2/s), while carbon residue is ten times below the European standard threshold.

In the last years, the relationship between biodiesel fatty acid composition and several biodiesel chemical and physical properties has been demonstrated [23, 55, 56]. SFWO composition, as previously mentioned, showed a fatty acid profile rich in C18:1, C18:2 and C16:0 acids, with length of chain (LC) and unsaturation degree (UD) values close to optimal values (LC=16.96 and UD=1.16, according to Pinzi *et al.* [44]). Authors found a significant correlation between biodiesel LC and UD values and CN. Considering the mathematical model proposed by authors, the theoretical value of SFWOME CN was calculated (62.42). This value is higher than that from commonly used biodiesel, i.e. soybean oil biodiesel (52.62) or sunflower oil biodiesel (53.90). LCV provides information about fuel energy that may be transferred during combustion [44]. Experimental values (Table 5) showed similar values to previously transesterified vegetable oils (sunflower, soybean, rapeseed or palm oil biodiesel) assisted by US [41].

SFWOME oxidation stability does not fulfill European standard threshold. Using the model developed by Park et al. [57], based on fatty acid composition, an oxidation stability value of 7.41 h was predicted, whereas, experimental value shows poorer result (around 4 h, see Table 5). This may be due to the presence of free radicals in SFWO, as a consequence of cooking processes. Considering used frying oils, Chuah *et al.* [58] demonstrated that heating process increases peroxide values, while Jain and Sharma found that presence of light, air and metals (metal content shown in Table 5) can induct parallel reactions [59]. In this sense, Varatharajan and Pushparani [60] established that addition of 1000 ppm of phenolic antioxidants, i.e. pyrogallol (PY), tertiary-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) may improve oxidation values [61].

Table 4.2-5. Quality parameters of optimal biodiesel provided by both kind of transesterifications[5].

QUALITY PARAMETER	TEST METHODS	CT biodiesel (1 h)	UT biodiesel (20 min)
FAME (%, w/w)	EN 14103; Min: 96.50	93.3 ± 0.67	93 ± 1
Linolenic ester content (%, w/w)	EN 14103; Max: 12	1.1 ± 0.10	1.1 ± 0.096
Free glycerol (%, w/w)	EN 14105; Max: 0.02	0.83 ± 0.05	0.73 ± 0.04
Monoglycerides (%, w/w)	EN 14105; Max: 0.7	0.7 ± 0.18	0.44 ± 0.09
Diglycerides (%, w/w)	EN 14105; Max: 0.2	0.3 ± 0.15	0.22 ± 0.08
Triglycerides (%, w/w)	EN 14105; Max: 0.2	0.7 ± 0.1	0.6 ± 0.1
Na^+ (ppm)	EN 14538; Max: (Na+K) = 5 mg/kg (Ca + Mg) = 5 mg/kg	5.10 ± 0.13	5.20 ± 0.09
K^+ (ppm)		0.69 ± 0.05	0.67 ± 0.05
Mg^{2+} (ppb)		0.082 ± 0.024	0.081 ± 0.015
Cu^{3+} (ppb)		1214.00 ± 26.34	1205.00 ± 25.43
Kinematic viscosity at 40ºC, ν (mm^2/s)	EN ISO 3104; 3.5-5.0	4.10 ± 0.07	4.23 ± 0.07
Low calorific value[1], LCV (J/g)	ASTM D240; Min: 35000	39454 ± 103	39572 ± 61
Cold filter plugging point (ºC)	EN 116	-4	-3.7
Water content (ppm)	EN ISO 12937; Max: 500	249 ± 48	380 ± 96
Density at 15ºC, ρ (kg/m^3)	EN ISO 3675; 860-900	870 ± 1	880 ± 1
Flash point (ºC)	EN ISO 3679; Min: 101ºC	166 ± 1	163 ± 3
Carbon residue (% w/w)	EN ISO 10370; Max: 0.30	0.03 ± 0.02	0.03 ± 0.02
Oxidation stability at 110ºC (h)[1]	EN 14112; Min: 8	2.10 ± 0.06	3.25 ± 0.08
Cetane number[2]	EN 5165; Min: 51	62.42 ± 0.64	62.42 ± 0.64
Acid value (mg KOH/g)	EN 14104; Max: 0.50	0.16 ± 0.01	0.17 ± 0.01

[1]ICP: Inductively Coupled Plasma Mass Spectrometry
[2]Calculated following Pinzi et al [44], threshold calculated following EN 5165

European standard controls vegetable oil-based biodiesel metal concentration. However, the raw material used in this work comes from very different origins and presents storage conditions prone to the appearance of other metals (i.e. copper) which

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[5] CT: conventional transesterification; UT: ultrasound assisted transesterification

are not contemplated in the standard. In addition, enzymes and microorganisms can deteriorate oxidation stability during the whole process, from raw material production to biodiesel storage [62].

Fuel cold properties (below -3ºC) are in agreement with European limits. In this sense, low CFPP values (under cold climates) may lead to potential filter obstruction and subsequent compression-ignition engine problems [62]. European standard provides recommended values considering region climate. In this work, considering EN 590, biodiesel could be classified as adequate for its use in temperate climate. Finally, flash point (FP) shows values over minimum standard threshold, while those for water content and density are below maximum limits

Conclusions

Solid food waste oil (SFWO) was extracted from food waste and its conversion in biodiesel was optimized by both conventional heated-stirred reaction and using ultrasound (US) as auxiliary energy. SFWO showed a singular fatty acid composition suitable for biodiesel production. However, the presence of free fatty acids involves the need of acid-catalyzed esterification pre-treatment (prior to basic-catalyzed transesterification) making the process more expensive by adding one more step. Compared with conventional method, US-assisted reaction has provided significant advantages in reaction time and energy savings. Physical and chemical property analysis showed that biodiesel fulfil European biodiesel standard, excepting oxidation stability, FAME yield and glyceride content. For this reason, this biofuel can be used as fuel by blending with diesel fuel or by adding phenolic antioxidants

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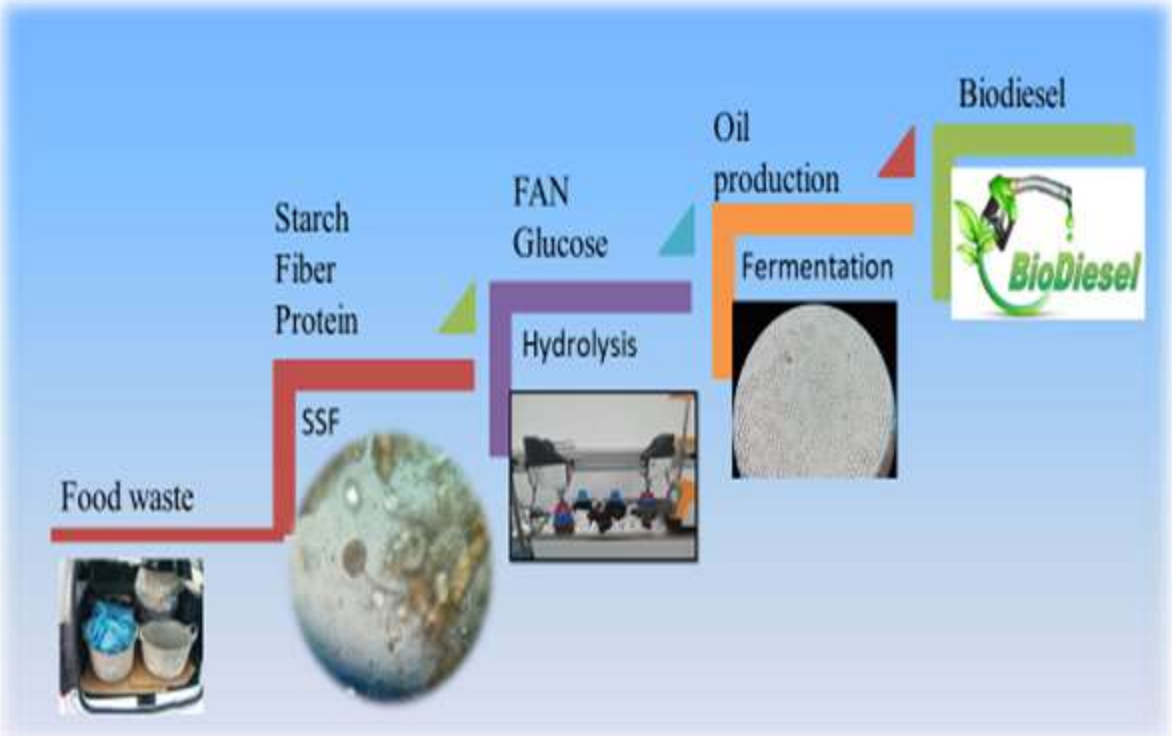
CHAPTER 5.

VALORIZATION OF FOOD-INDUSTRY WASTES THROUGH A BIODIESEL BIOREFINERY APPROACH



Abstract

Current environmental predicament requires the search for renewable and sustainable alternatives to fossil fuels, mainly in the transport sector. Biodiesel may replace diesel fuel under an environmentally friendly and economically efficient process, as long as the raw material employed is low-cost and derived from sustainable sources. Such alternative feedstock, with no competence in food markets or for land uses, could be provided by microbial oil produced by oleaginous microorganisms. Chemical composition of microbial oil varies depending on cultivation parameters (i.e. fermentation substrate or growing conditions) and it might be similar to that of the most commonly used vegetable oils in biodiesel industry. Considering that oleaginous yeasts may accumulate intracellularly lipids, via hospitality food waste fermentation, this work focuses on the valorization of residues from food service sector, to produce biodiesel within the biorefinery concept. In light of starch and protein content of food waste (FW), controlled enzymatic hydrolysis was considered feasible, and required enzymes were provided through solid state fermentation using A. awamori and potato peel as substrate. Values for proteolytic and glucoamylase activities of 50 and 30 U/g, respectively, were achieved. Further hydrolysis, under different FW concentrations, temperatures and reaction times was carried out, reaching around 98% conversion of starch into glucose. Crude hydrolysates were subsequently used in shake flask fermentations, with oleaginous yeast *Rhodosporidium toruloides* Y-27012, attaining a total dry weight (TDW) of 32.9 g/L and a lipid content within cells of 36.4 %. Results of *R. toruloides* fermentations in fed-bath bioreactor showed a TDW of 53.9 g/L and a lipid production of 26.7g/L. Moreover, fatty acid profile of microbial oil from bioreactor samples showed palmitoleic acid (C16:0) and oleic acid (C18:1) as main components, both added up to a value close to 75 % of the total fatty acid content. The principal component analysis showed a fatty acid profile similar to both soybean and solid food waste oil. Finally, microbial oil was transesterified to biodiesel, achieving a yield of 94 %, which almost fulfills European biodiesel standard EN 14214 . Results from this work show that valorization of food-industry wastes is feasible within a sustainable biorefinery concept focused on biodiesel as main product.

5.1. Introduction

Mankind’s current and future challenges depend on our capacity to provide solutions to reach a balance between our needs and the availability of natural resources of a finite planet. Over the last sixty years, World human population has tripled from 2.5 billion, in 1950, up to 7.3 billion, today. The United Nations Department of Economic and Social Affairs (UN DESA) predicts an increase up to 9.5 billion by 20401. This exponential population growth trend may be due to technical advances that have increased life expectancy, among other causes. In fact, over the last 100 years of industrialization, production capacity of goods and services has improved, resulting in an increase in purchasing power, health benefits and life quality for the population of industrialized world. Optimization of agri-food industry has allowed a popular access to hygienic and quality food. The Green Revolution (1950 to late 1960s) increased agricultural production worldwide. Since then, food manipulation processes have been continuously improved and mechanized, while a global market has been established. However, the above-mentioned population growth and economic improvements have been achieved at the expense of high energy and water demands, waste generation and, therefore, significant environmental impact. Current water demand has increased to unsustainable levels; thus, water consumption for human and animal needs, including industry and land irrigation water demand, has increased in direct proportion with human population and our quality of life, although, available fresh water is the same than 3000 years ago. Also, deforestation has increased soil erosion and, subsequently, accelerated climate change2. Current consumer disposal habits, together with agri-food industry residues, are responsible for around 1.3 billion t/year of food waste (FW), which is more than half of the food produced worldwide3. Another important concern for the next 20 years has to do with energy demand, that accounted for 274 million barrels of oil equivalent per day (mboe/d) in 2015, while the Organization of the Petroleum Exporting Countries (OPEC) estimates a demand of 365 mboe/d in 2040, with an average annual growth of 1.2 %4.

Over the last century, petrochemical industry has improved our daily life with multiple applications and generation of direct and indirect employment. In the primary energy sector, more than 30% of consumed energy is fossil oil-based one and this figure rises to over 94% in the transport sector. Current global fossil oil demand for energy is expected to grow from 1.2 to 1.4 mb/d within the next ten years. Yet over medium to long term a drop off in demand is expected; by 2024, this will fall to 1 mb/d4, due to the ecological transition and decarbonization tendency. Nevertheless, fossil oil is also used as a chemical base to produce a large number of products, and its derivatives are present in numerous sectors, i.e. agri-food, cosmetics, construction, painting, lubricant, among others. According to this, the International Energy Agency (IEA) goes further and predicts an increase of total fossil oil demand to 1.7 mb/d5 in the next ten years. As it is already well known, the use of fossil oil presents many negative impacts, i.e. environmental degradation, human health damage and geopolitical instability, that call into question the use of this resource.

Biorefineries may play a significant role in the necessary energy transition process, from traditional petroleum refineries to a sustainable model. In line with IEA recommendations, biorefineries must develop efficient processes in which renewable biomass is transformed into chemicals, materials, power or heat. To meet circular economy basis, with an important role in decarbonizing primary energy and transport sectors, the initial biorefining stage (raw material acquisition) must follow a recovery or recycling process. Eventually, biorefineries will represent a crucial mainstay for future waste management procedures, specifically for organic material sorted as FW.

Official organizations and researchers are focused on the reduction of edible food losses, yet FW comprises both, edible and non-edible food discarded throughout food chain, including primary, manufacturing, logistic and storage sectors, and also post-consumer steps6. According to its chemical profile, FW can be considered a rich raw material for biorefineries, thus it is composed of macronutrients, i.e. proteins, starch, lipids and fibre (20.7, 27.9, 25.5, 20% w/w db, respectively) as well as trace elements, including Fe, Ni, Cu or Mn. A biorefinery may, therefore, use FW to produce bioenergy, high-value-added products (e.g. antioxidants, such as polyphenol) and bioplastics, following hydrolysis and further fermentation processes7.

In fact, an attractive product provided by biorefineries is biodiesel, which can substitute conventional diesel fuel (straight or blended with diesel fuel) without significant engine modifications. However, after a growth in biodiesel production of 6.5% over the last 15 years, with a total production in 2016 of 36 billion liters, current production is tending to decrease due to raw material prices, or new energy policies which have been proposed in international conferences, i.e. the United Nations Climate Change Conference (COP21) which was held in Paris. Most likely, over the next decades, there will be a transition in the automotive sector towards electric engine-based cars due to the need for reduction in exhaust gas emissions in cities, However, biodiesel is an essential component of future low carbon energy system of maritime, agricultural, aviation and transport sector, whose demand for fuel will certainly grow in the next 40 years. The aviation sector demand for fuel was 6.3 mb/d in 2017 and is expected to reach 9 mb/d in 2040. Likewise, the heavy duty vehicle sector, which is forecast to increase by around 4.1 mb/d from 2017 to 2040, will rise its fuel consumption from 43.6 mb/d to 47.8 mb/d4.

In this context, FW recovery represents a promising alternative for the future energy supply. On one hand, according to Yang et al.8, solid food waste oil (SFWO) can be extracted and directly transesterified, while remaining macronutrients (i.e. protein or starch) may be used as low-cost fermentation culture substrate for microorganism growth. On the other hand, oleaginous yeasts are microorganisms able to accumulate intracellularly lipids in proportions above 70% (w/w). Examples reported in the literature with Cryptococcus curvatus ATCC205099, Cryptococcus curvatus MUCL 298199, Rhodotorula glutinis TISTR 51599, Yarrowia lipolytica10, Prunus domestica11, Candida pulcherrima11, Candida oleophila ATCC 201779, Malus domestica11 or Rhodosporidium toruloides AS 2.13899, DSM 4449, Y49 and 2116712 show excellent lipid content and productivity in fed-batch bioreactor. Some advantages of this yeasts compared to conventional vegetable oils are a short life cycle, less labor required and independence of climate and year season13. According to Koutinas et al.14, microorganisms used as cell factories may be the central strategy for a sustainable development of biodiesel and other oleochemical products.

In consequence, two key factors must be considered in the production of microbial oil (MO) as raw material for biodiesel production: firstly, its fatty acid profile, that will define biodiesel properties; secondly, lipid content in microbial cells, that will decide its profitability. Both aspects also depend on fermentation conditions and substrate used. Maina et al.11 tested several carbon substrates (glucose, xylose, galactose, mannose and arabinose) in fermentations with three different microorganisms, proving dissimilarities in fatty acid profiles and, therefore, in biodiesel properties.

Fatty acid profile of MO mainly contains C16 and C18 fatty acids, similar profile to animal and vegetable oils, due to the lipogenesis mechanisms which have been conserved from bacteria to humans15. Efficiency of microbial oil production may be improved through metabolic engineering, consisting on modifications of the metabolic pathways in microorganisms, to optimize cost-effectiveness ratio at an industrial scale16. In any case, all commercial biodiesel must meet European biodiesel standard EN 14214 or the American Society for Testing and Material Standard (ASTM) D6751, that regulate biodiesel quality in Europe and States, respectively.

Manufacturing cost is a decisive factor in the production of MO. In 2008, production cost was calculated as 3 US$/l17 while current price is close to 2.4 US$/l18 and continues to follow this descending trend. One critical point to improve economic competitiveness of the process, over the last ten years, has been the reduction of raw material costs, and this includes the use of FW as feedstock for MO production, replacing expensive commercial substrates. For example, Tsakona et al.19 used a flour-rich waste hydrolysate to produce MO from L. starkeyi, reporting a total dry weight (TDW) of 109.8 g/L and an oil content of 57. 8 % (w/w). Moreover, Johnravindar et al.20 showed also interesting results using leachate from FW with Cryptococcus curvatus (TDW 12 g/L; lipid content 20%), Rhodotorula glutinis (TDW 12.5 g/L; lipid content 28%) and Yarrowia lipolytica (TDW 20.9 g/L; lipid content 49 %).

The main objective of this study is the valorization of FW residues from the hospitality sector to produce bioenergy, following circular economy principles. For this purpose, MO biodiesel from oleaginous yeast Rhodosporidium toruloides Y-27012, using FW from the hospitality sector as substrate will be produced and optimized, aiming to increase economic competitiveness. Methodology will comprise a two-step process for the transformation of biopolymers present in FW (e.g. protein and starch) into monomers. Firstly, through solid state fermentation (SSF) with Aspergillus awamori, an enzyme-rich consortium will be produced and added, in a second step, to a FW suspension for further hydrolysis. The result of this process will be a generic fermentation feedstock that may be used, in replacement of commercial supplements, for fermentation cultures. This FW substrate will be evaluated in shake flasks and fed-batch bioreactor for oil production. Extracted oil will be transesterified and FAME will be analyzed according to European biodiesel standard EN 14214.

5.2. Materials and methods

Figure 1 shows a schematic description of the sampling, characterization and conversion processes applied to FW samples

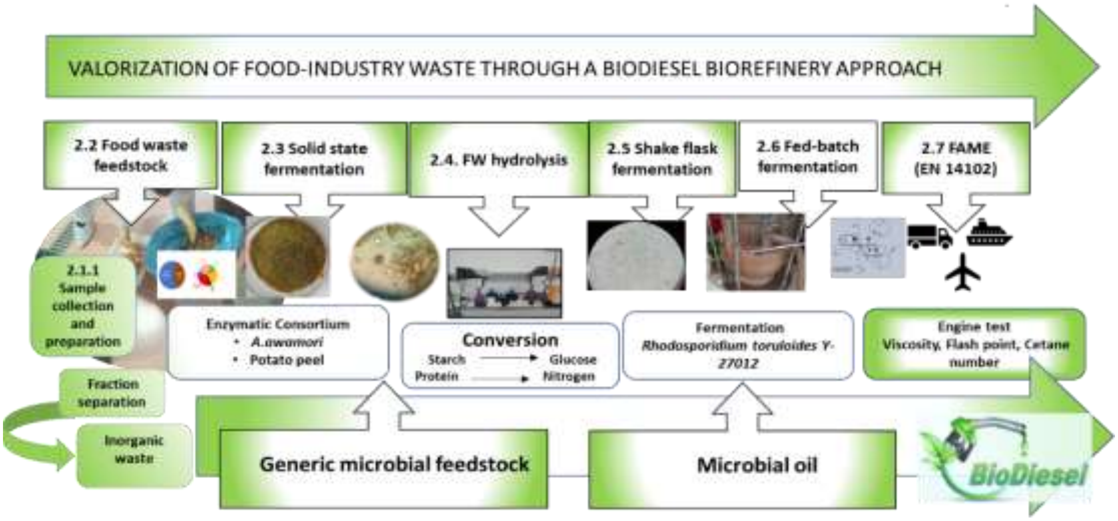


Figure5- 1. Valorization food-industry waste through a biodiesel biorefinery approach.

5.2.1 Microorganisms and media

A. awamori 2B.361 U 2/1 was kindly supplied by Professor Colin Webb (University of Manchester, UK) and used for SSF. Fungal spores were stored at 4ºC in slopes containing wheat bran (WB) (5% w/v) and agar (2% w/v).

Oleaginous yeast strain R. toruloides Y-27012 was used in liquid fermentations for MO production. Yeasts were stored at 4ºC on both agar slopes as agar petri dishes, containing glucose (10 g/L), peptone (10 g/L), yeast extract (10 g/L) and agar (20 g/L). Inoculum was prepared in liquid medium containing glucose (10 g/L), peptone (10 g/L) and yeast extract (10 g/L) and cultivated for 24 h at 28ºC in a rotatory shaker (ZHHWY-211C Series Floor Model Incubator, PR, China) at 180±5 rpm agitation speed .

Potato peel (PP), supplied by an industrial potato chip producer from Cordoba, was used as substrate for SSF of A. awamori. In a previous step, samples were dried at 70ºC for 24 h in an oven (Incudigit 36 L, Selecta, Spain) until 2% (w/w) humidity content21. SFW was used as hydrolysis substrate to produce a generic fermentative medium. SFW was collected from four local restaurants in Cordoba (Spain): the University campus cafeteria (UCC), an Italian restaurant (IR), a fine dining restaurant

(FDR) and a grill restaurant (GR). Samples were blended and homogenized using an industrial mill (AM80ZBA2YH, AEG, Germany). Finally, milled samples were lyophilized for three days in a lyophilizer (Telstar 55, LyoQuest, Spain) and stored at -204°C . Oil was extracted from lyophilized solid samples by Soxhlet method and used for biodiesel production22. After oil extraction, FW samples were analyzed and main components quantified, as reported in a previous Chapter, were: ash (7.2%, w/w), fiber (13%, w/w), protein (25% w/w) and starch (34.9% w/w).

5.2.2 Solid state fermentations

Crude enzyme production by fungal strain *A. awamori* was carried out in 250 mL Erlenmeyer flasks containing 5 g of dried potato peel, previously sterilized at 121ºC for 20 min. The inoculum used consisted on a fungal spore suspension with an initial concentration of 2 10^6 spore/mL. Inoculum preparation was performed by sporulation of *A. awamori* on solid media containing WB (5% w/v) and agar (2% w/v) in 250 mL Erlenmeyer flask, for 5 days and 30ºC, following Kachrimanidou et al.[15]. Optimal enzymatic activities for glucoamylase, protease and invertase were evaluated at varying initial moisture contents by adding tap water (50, 55, 60, 65 and 70 % w/w), PP particle size (by using a blender and a sieve with three different sizes, namely 1, 2 and 3 mm) and fermentation duration. All experiments were performed by triplicate for statistical analysis.

5.2.3 Production of food waste hydrolysate

Resultant fermented solids from SSF were macerated in a kitchen blender with 500 mL sterilized tap water creating an enzyme-rich suspension. Production of food waste hydrolysate (FWH) was performed by mixing this suspension with certain amounts of FW in 1 L Duran bottle. Experiment conditions were uncontrolled pH and continuous agitation (using magnetic stirrers) at varying temperatures, initial solids and enzymes concentrations, and process duration. First set of experiments was carried out varying hydrolysis temperatures (40, 45, 50, 55, 60 and 65ºC), with 75 g/L (w/w) FW, initial glucoamylase and proteolytic activities of 0.95 and 1.6 U/mL, respectively, for 60 h.

Subsequently, optimal initial FW concentration and enzymatic activities were evaluated by using 50, 100, 150 and 200 g/L of FW, as well as two initial glucoamylase (0.94 and 1.6 U/mL) and proteolytic activities (1.24 and 2.1 U/mL). To optimize the duration of the hydrolysis process (90 h), a final set of experiments, based on previous results, was performed. All successive hydrolysis experiments were performed according to optimal parameters, namely FW initial concentration (200g/L), initial glucoamylase and proteolytic activities of 1.6 and 2.1 U/mL, respectively, and 35 h. All experiments were performed by triplicate.

Samples were collected at random intervals of two hours and centrifuged at 12,000g for 20 min in a Hettich Universal centrifuge model 320-R (United Kingdom). Supernatant was filtered and kept for glucose, inorganic phosphorous and free amino nitrogen (FAN) determination. The liquid medium obtained under optimal hydrolysis conditions was filtrated, using a 0.2μm–filter-sterilizing unit (Polycap^{TM}AS, Whatman Ltd, Germany), and its pH was adjusted to 6 for further fermentation process with *R. toruloides.*

5.2.4 Shake flask fermentations

Fermentations were carried out in 250 mL Erlenmeyer flasks, with a working volume of 50 mL of FWH inoculated with 1 mL of a 24 h exponential pre-culture of *R. toruloides*, containing commercial glucose, yeast extract and peptone. An initial set of fermentations with *R. toruloides*, using three different C/FAN ratios (60, 120 and 180 w/w) that were adjusted through FWH dilution with sterilized water was carried out. A pH constant value of 6 was achieved by manual addition of 5M NaOH and 5M HCl dilutions to the cultivation broth. Optimal C/FAN ratio was determined and used for a final set of experiments in the presence of a trace elements solution composed of KH_2PO_4 (7 g/L), Na_2HPO_4 (2.5 g/L), $MgSO_4\_7H_2O$ (1.5g/L), $FeCl_3\_6H_2O$ (0.15g/L), $ZnSO_4\_7H_2O$ (0.02 g/L), $MnSO_4\_H_2O$ (0.06 g/L) and $CaCl_2\_2H_2O$ (0.15 g/L). Flasks were incubated at 30ºC in previously described rotary shaker, at 180±5 rpm, until carbon source depletion. Every experimental point was carried out in duplicate, and the whole flask content was used for each sample, to ensure the reliability of MO quantification. Moreover, all parameters were analyzed in triplicate.

5.2.5 Fed-batch bioreactor fermentations

Fed-batch fermentation experiments were performed in a 3.5 L bioreactor (Infors 4, Switzerland) with a working volume of 1200 mL using FWH as nutrient source. pH was automatically fixed to 6 ± 0.1 using 5M NaOH and 5M HCl solutions. Initial medium was filter sterilized and inoculated with 10% (v/v) of overnight cultivated *R. toruloides* culture using commercial nutrient supplements. Two different sets of experiments were carried out, both with an initial glucose concentration of 60g/L, supplemented with the trace elements solution described in the previous section, at varying initial FAN concentration (800 and 400 mg/L). Fermentations were carried out in batch mode, until glucose concentration was less than 20 g/L. At this point, glucose concentration was maintained at 20 g/L by the automatic addition of a 500 g/L glucose solution (fed-batch mode). Samples were collected at random intervals of two hours (until fermentation was completed) and centrifuged at 12,000g for 20 min in previously mentioned centrifuge. Yeast cells were dried for TDW calculation and supernatant was filtered and kept for glucose, inorganic phosphorous and free amino nitrogen (FAN) determination. All parameters were analyzed in triplicate.

5.2.6 FAME transesterification

Fatty methyl ester (FAME) production was carried out through a two-step (basic and acid) transesterification reaction. Sodium methylate (0.5 M, Panreac®, Spain) was firstly used as basic catalyst, followed by HCl (37% purity, Scharlab®, Spain); both mixed with methanol (99% purity, J.T. Baker®) in a molar ratio solution of 1:9.5. In both cases, reaction conditions were 20 min, 70ºC and 400 rpm; reaction took place in a water bath, with a reactor equipped with reflux column, with phenolphthalein (99% purity, ACS reagent from Sigma- Aldrich) as indicator.

5.2.7 Analytical methods

5.2.7.1 Potato peel characterization

PP was dried at 105ºC, until constant weight, in an oven (Incudigit 32 L Selecta, Spain) and moisture content was calculated by weight difference. For ash content determination, samples were calcinated at 550ºC in an electric muffle (Select-Horn-TFT, Selecta, Spain) and ash percentage was calculated by weight difference. Total starch content was analyzed using a *Total Starch Assay Kit* (Megazyme®, US) according to AOAC 76.1322, while fibre content was analyzed following AOAC official methodology 973.18. Protein was calculated by means of *Total Kjeldahl Nitrogen* (TKN), following the AOAC method 955.04. It was estimated as 6.25 times the obtained TKN value using a Kjeldahl automatic (pro-Nitro M distiller, Selecta, Spain).

5.2.7.2 Enzymatic activity

Solid state fermentation was characterized through enzymatic activity analysis of glucoamylase, protease and invertase. Glucoamylase activity test was carried out by measuring glucose released during hydrolysis of 20 g/L (db) of pure starch (previously gelatinized at 75ºC for 20 min) in a phosphate buffer 0.2 M (pH 6), at 55ºC, over 15 min. Reaction was totally stopped using a 5% trichloroacetic (TCA) solution (ratio 1:1). One unit (U) of glucoamylase activity may be established as the quantity of enzyme that releases 1 mg of glucose in one minute, under test conditions.

Proteolytic activity test was carried out by FAN concentration measure, through hydrolysis of 15 g/L (db) of casein in a phosphate buffer 0.2 M (pH 6), at 55ºC, over 30 min. Reaction was stopped with a 5% TCA solution (ratio 1:1). One unit of proteolytic activity can be defined as the quantity of enzyme that releases 1 µg of FAN in one minute, under reaction conditions.

Invertase test was carried out by measuring the glucose released during hydrolysis of 10 g/L (db) of sucrose, in a mix of phosphate buffer 0.2M with citric acid 0.1 M (pH 5) at 50ºC, over 30 min. Reaction was stopped with 5% of TCA (ratio 1:1).

5.2.7.3 Supernatant characterization

Supernatants from SSF, shake flask and fed-batch fermentations were characterized in terms of sugar, inorganic phosphorous and nitrogen concentrations. FAN was analyzed following the *Ninhydrin Colorimetric Method* (according to the European Brewery convention). Inorganic phosphorous (IP) analysis was carried out according to the *Ammonium Molybdate Spectrophotometric Method*. Sugar was analyzed by HPLC, with an Aminex HPX-87 column (Biorad, Germany) and a differential refractometer (RI Waters 410, Germany), following the protocol proposed by Kachrimanidou el al[15].

5.2.7.4 Yeast cell characterization

After flask and bioreactor fermentation experiments, yeast cells and liquid fraction were separated by centrifugation at 12,000g for 10 min. TDW was analyzed by weight difference after drying fermentation biomass at 105ºC, for 24h. MO was extracted with a chloroform/methanol mixture (2:1 ratio), following the Folch method. Remaining solids were cold centrifuged at 9,000g and 4ºC for 10 min and solvent was removed by evaporation. MO concentration was calculated by weight difference.

FAME profile was analyzed by gas chromatography using a Perking Elmer GC Clarus 500 (Waltham, Ma, US) equipped with a capillary column of 30 m x 0.25 mm, 0.25 µm particle diameter and flame ionization detector FID (Perking Elmer, Germany).

Carotenoid extraction was carried out by adding 20 mL of Folsh solution, containing 20 mg of butylated hydroxytoluene (BHT, Sigma-Aldrich) as antioxidant agent, to lyophilize sample that was incubated in dark for exactly 3 days. Subsequently, solvent was removed by vacuum evaporation. Carotenoids were extracted by adding a specific quantity of light petroleum ether (boiling point 40–60ºC), followed by a filtration step using 0.22 µm membrane filter. Finally, sample absorbance was provided by a spectrometer (UV- 2000 Hitachi, Paris, France) at 444 mm, using distillate water as blank. Total carotenoid concentration was expressed as µg of β-carotene equivalent per gram of TDW, after the following equation.

Total carotenoids (µg/g) = 1000 A V/ (E W)

Where:

A= absorbance; V =volume of petroleum (ml); E= 1% extinction coefficient (2592) and W = TDW (g/L).

5.2.7.5 Biodiesel characterization

According to EN 14103 standard, FAME yield was measured using previously mentioned gas chromatrograph, coupled to a flame ionization detector and comprising a 30 m × 0.25 mm Elite 5-ms (0.25 µm particle diameter) capillary column.

Water content was measured after EN ISO 12937 standard in a Karl Fischer titrator (DL32 Mettler Toledo, US). Oxidation stability was carried out by EN 14112 standard, using a professional biodiesel Rancimat equipment (Metrohm, Switzerland). Carbon residue (CR) analysis was performed in a micro carbon residue tester (Alcor MCRT-160, PAC, US) following EN 10370 standard. Higher calorific value (HCV) was analyzed using an IKA bomb calorimeter C200 (Staufen, Germany) and following ASTM D240 standard. Kinematic viscosity (ν) was determined according to EN ISO 3104, in a glass capillary-type viscometer (Cannon-Fenske, size 150). Acid value (AV) was performed according to EN 14104 standard. Flash point (FP) was carried out in a Seta Flash (series 3 plus, Instrumentación Analítica S.A., Spain) following EN ISO 3679. Finally, cetane number (CN) and could filter plugging point (CFPP) were calculated according to Pinzi et al[23] expression.

5.2.7.6 Statistical analysis

Principal component analysis (PCA) was carried out using IBM SPSS statistical software (NY, US).

5.3. Results and discussion

The proposed biorefinery process begins with the production of a generic fermentation feedstock, in two steps: firstly, potato peels were used as substrate for SSF with *A. awamori*, to produce hydrolytic enzymes (glucoamylase, protease and invertase) as well as additional nutrient supplements. Remaining fermented solids, together with a certain amount of lyophilized FW, were subjected to hydrolysis, so starch and protein were torn into smaller blocks (*e.g.* glucose and amino acids). Supernatant (generic fermentation feedstock) was used in shake flask and bioreactor fermentations with *Rhodosporidium toruloides* for the production of MO, which was subsequently transformed into biodiesel. A complete overview of the described FW valorization process is presented in Figure 1.

5.3.1 . Potato peel substrate

The first step in this study was to find out a suitable and homogeneous raw material to be used as substrate in SSF with *A. awamori*. PP appeared as a good candidate, thus more than 30% (w/w, db) of potato production is lost in manufacturing processes, which represents above 4 million tons of solid waste, in 2017, worldwide[24]. PP has relatively high fibre and starch contents (65.3% and 38.1% w/w, respectively) while its protein content is much lower (only 3.9% w/w). According to Table 1, that shows a comparison between PP and other raw materials used as substrate for SSF, PP has a fibre content similar to babassu cake and a starch concentration similar to wheat straw, while its protein content is lower than all raw materials reported in the table. SSF process is sensitive to minor C/N variations, thus this factor plays an important role in the balance between fungal growth and exogenous enzyme production. Indeed, Table 1 shows important variations in proteolytic activity when *A. awamori* is fermented on substrates with different protein contents, and therefore, low proteolytic activity for *A. awamori* cultivated on PP may be expected.

Table 5-1. Comparison of different organic substrates used for solid state fermentation

Raw material	Moisture	Protein	Starch	Carbohydrates	Oil	Fibre	Microorganism	Glucoamylase	Protease	Reference
	% (w/w)							U/g		
PP[7]	80.7±0.7	3.9±0.1	38.04±1.5	n.d	n.d	65.2±2.1	*A. awamori*	n.d	n.d	This study
WMB[1]	9.7	20.0	12	n.d.	n.d.	n.d.	*A. awamori*	20.0	233.0	[24]
SFM[2]	3.7	26.6	n.d.	n.d.	0.9	19.5	*A. orizae*	n.d.	400.0	[19]
BC[3]	10.0	18.8	n.d.	9.3	n.d.	49.5	*A.awamori*	105.0	n.d	[29]
WB[5]	9.5	12.3	38.8	n.d.	n.d.	n.d	*A. awamori*	25.0	50.0	[30]
WB[5]	9.5	12.3	38.8	n.d.	n.d.	n.d.	*A. awamori*	301.2	170.3	[31]

1: Potato Peel; 2: Wheat milling by-product, 2: Sunflower meal; 3: Babassu cake; 4: Wheat straw; 5: Wheat bran, n.d.: no data

5.3.2 Solid state fermentation of FW

According to the composition of collected FW samples (rich in starch and protein), fungal strain *A. awamori* was selected to produce an enzymatic consortium able to hydrolyze them. Initial enzyme targets were glucoamylase, proteolytic and invertase activities. First set of experiments was carried out at different initial moisture contents and 30ºC (optimal SSF temperature, according to Tsakona et al.[19]) and results are shown in Figure 2. It can be appreciated that proteolytic and glucoamylase activities describe a different behavior than invertase activity: while the highest activities for glucoamylase (28.2 U/g) and protease (53.6 U/g) were found using a substrate with 65% of initial moisture content, invertase (20 U/g) required only 55% of initial moisture content. As a result, 65% of initial moisture content was selected as optimum, thus invertase activity was relatively low and, therefore, discarded as target enzyme. In the following experiments, only proteolytic and glucoamylase activities were optimized.

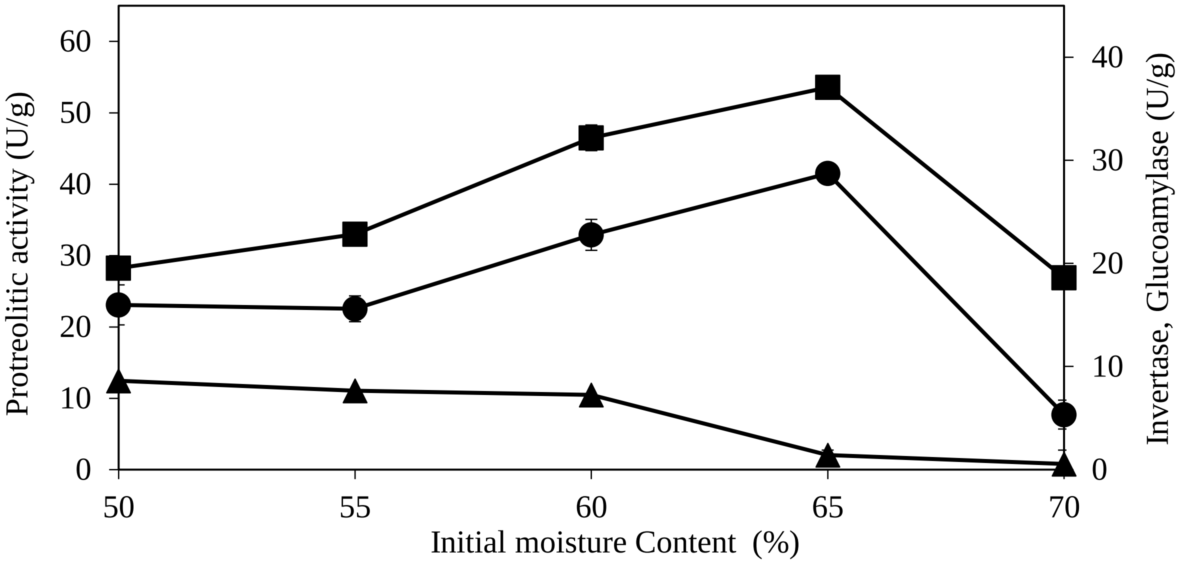


Figure 5-2. Effect of initial moisture content at 30ºC in proteolytic activity (■), glucoamylase activity (●) and invertase activity (▲) during SSF.

Among parameters affecting SSF, substrate porosity and particle size are related to fungal access to nutrients, oxygen, carbon dioxide diffusion and moisture[28]. A second set of SSF experiments was carried out using three different substrate sizes (1, 2 and 3 mm), at 30ºC and 65% initial moisture content, over 98 h. Results reported in Figure 3 show how enzymatic activity increases as particle size is reduced, achieving a maximal enzymatic activity (52.3 U/g proteolytic activity and 30 U/g glucoqmilase) for 1 mm particle size. These results are in line with literature. In this sense, Kantifedaki et al.[29] reported high pigment synthesis when *Monascus purpureus* was cultivated in small orange processing waste substrate particles (<2mm).

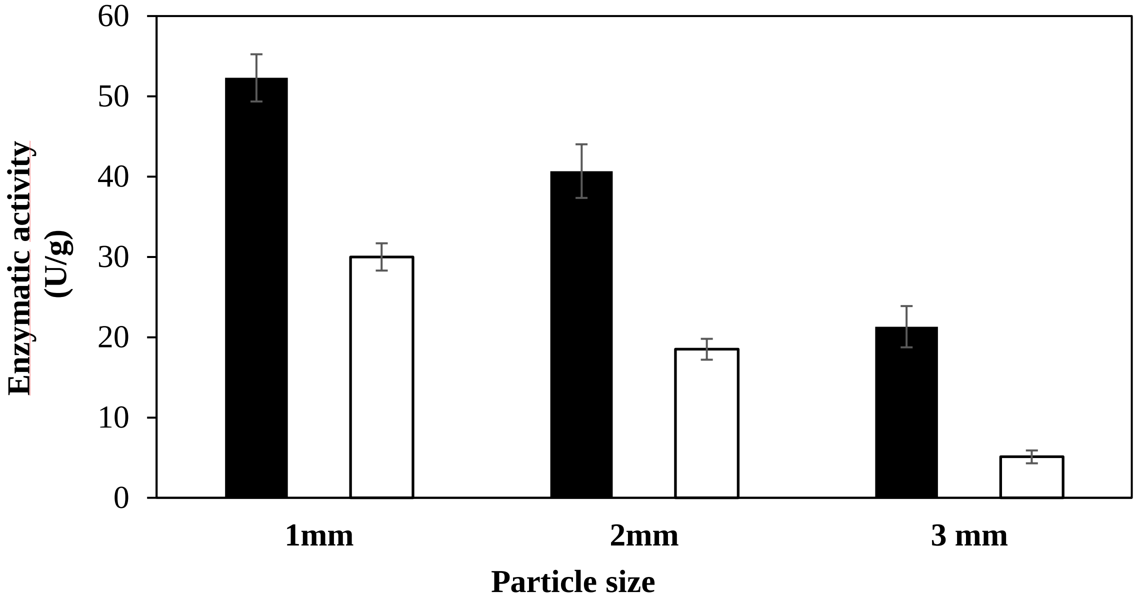


Figure 5-3. Effect of particle size in proteolytic activity (□) and glucoamylase activity (■) during SSF after 98 hours.

A final set of experiments was carried out at 30ºC, 65% (w/w, db) of initial moisture content, 1 mm substrate particle size and at varying fermentation times. According to Figure 4, highest activity values (30 and 52 U/g for glucoamylase and protease, respectively) were achieved after 65 h of fermentation. Experiments were completed after 90 h, when enzyme activities were gradually reduced, as reported in literature[19]. On one hand, glucoamylase activity high value (30 U/g) was consistent with that from other raw materials, i.e. wheat milling, wheat straw or wheat bran, as shown in Table 1. In this sense, Tsakona et al.[19] reported activities close to 20 U/g for SSF with wheat milling by-product (WMB) as substrate and *A. awamori*, while Wang et al.[26] reported a production of 40 U/g on wheat bran. Nevertheless, other studies, i.e. Castro et al[25], reported higher values for glucoamylase activity (107 U/g). On the other hand, proteolytic activity, as expected, presented lower values than those reported in literature (Tsakona et al.[19] exceeded 200 U/g using WMB) due to the low protein content in PP substrate.

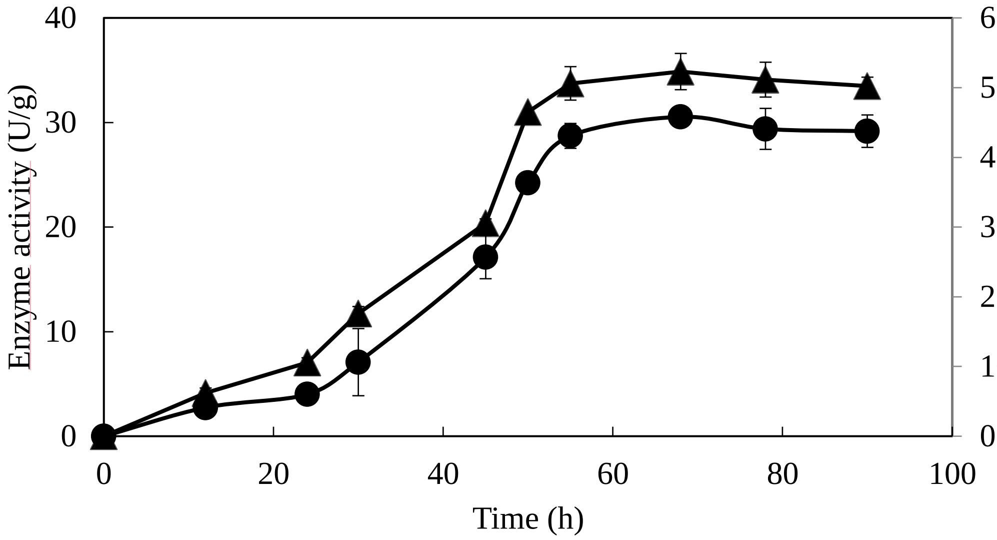


Figure 5-4. Time evolution in proteolytic activity (▲) and glucoamylase activity (●) during Solid state fermentation

5.3.3 . Food waste hydrolysis

This step is focused on the hydrolysis of lyophilized FW samples, using an enzyme consortium generated during previous SSF. Table 2 presents results for starch to glucose and TKN to FAN conversion yields. The aim is the production of a nutrient-rich aqueous medium, composed by macromolecules from both, FW and remaining fungal cells from SSF. As mentioned in Materials and methods section, hydrolysis optimization took place through three sets of experiments. First set was designed to find optimal working temperature under the following conditions: uncontrolled pH, 75 g/L (w/w) initial FW concentration, 0.95 U/mL glucoamylase activity and 1.6 U/mL proteolytic activity. FW macromolecule hydrolysis at different temperatures (40, 45, 50, 55, 60 and 65 ºC) was monitored by glucose, FAN and IP concentration analysis, as shown in Figure 5. Moreover, Table 2 presents the starch to glucose, and TKN to FAN conversion yields. Optimal temperature range for starch to glucose conversion was found in the range from 50 to 60ºC, with peak value at 55ºC (22 g/L of glucose concentration and a saccharification degree of 72%). Glucose production, at temperatures below 50ºC, was 1.7 times lower than that at 55ºC, while at temperatures above 60ºC, enzymatic denaturation begins, as reported in the literature[30,31], and glucose decreases to 3g/L. FAN production presented a different pattern and the optimum yield was located in the range of 45ºC to 55ºC (3.1 and 3.9% yield conversion respectively), with a maximum at 50ºC (5.85%) and a FAN production of 350.56 mg/L. Optimal concentration of IP (700 mg/L) was found at 45ºC, declining for temperatures above this point: between 50 and 55ºC it reduced slowly, but above 55ºC reduction was more pronounced, while at 60ºC, IP concentration reduced twice as much as at 45ºC.

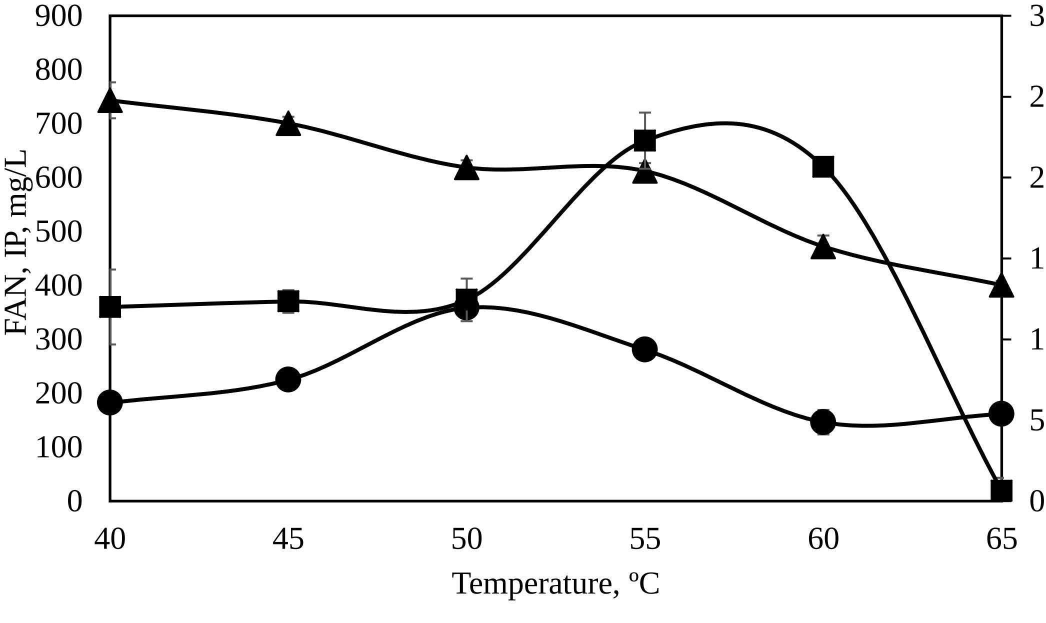


Figure 5. Effect of temperature on glucose (●), free amino nitrogen (FAN) (■) and inorganic phosphorous (IP) (▲) production during hydrolysis of 75 g/L FW solution with initial glucoamylase and proteolytic activities of 0.9 and 1.6 U/mL

Optimal hydrolysis temperature must provide a balance between nitrogen and glucose (C/N ratio) for optimal fermentation conditions. On the view of the above reported results, selected temperature was 55ºC, optimal for glucoamylase activity, even though it is not optimal for proteolytic activity, FAN concentration (1.27 times lower than the maximum found at 45ºC) or IP (600 mg/L).

Table 5-2. Starch and protein (TKN) conversion yield during hydrolysis at varying temperatures, Food Waste concentrations and initial enzymatic activities.

set one: temperature profile		
FW Concentration: 75 g/L	**Starch to glucose conversion yield (%)**	**TKN to FAN conversion yield (%)**
Temperature ºC	**Glucoamylase activity (U/mL)**	**Proteolytic activity (U/mL)**
	0.9	1.6
40	52.6 ± 0.1	3.7 ± 0.8
45	53.2 ± 0.1	3.1 ± 0.1
50	60.5 ± 0.1	5.8 ± 0.1
55	71.9 ± 0.8	3.9 ± 0.1
60	50.5± 0.5	2.4 ± 0.1
65	2.2 ± 0.1	1.9 ± 0.1

Table 2 (Continued) Set two: varying FW concentrations and initial enzymatic activities

Temperature: 55ºC	**Starch to glucose conversion yield (%)**		**TKN to FAN conversion yield (%)**	
	Glucoamylase activity (U/mL)		**Proteolytic activity (U/mL)**	
FW concentration (g/L)	0.9	1.2	1.6	2.8
50	55.1± 0.5	89.5 ± 0.5	2.8±0.1	3.28 ±0.01
100	67.6 ±0.1	90.1±0.2	3.5±0.3	4.71±0.01
150	77.6±1.2	91.5±0.1	4.5 ±0.1	9.0±0.2
200	84.4±1.2	97.6±0.2	3.2 ±0.1	11.9±0.5

The second set of experiments, carried out under uncontrolled pH conditions and 55ºC, was designed to find the effects of initial FW concentration and initial enzymatic activity on the hydrolysis yield. Considering possible side effects due to solution high viscosity, a solid maximal concentration of 200 g/L was adopted and experiments were, therefore, performed with 50, 100, 150 and 200 g/L of FW. Enzyme activities considered were 0.9 and 1.2 U/mL for glucoamylase and 1.6 and 2.8 U/mL for proteolytic activity. Optimum results, presented in Table 2 (97.6% conversion yield) were obtained for FW concentration of 200g/L (db), with initial glucoamylase and proteolytic activities of 1.24 and 2.08 U/mL, respectively. Glucose concentration reached a value close to 100 g/L, as shown in Figure 6, while protein to FAN conversion yield was significantly lower, with a final concentration of 600 mg/L. In this case, a 1.3-fold increase in the initial activity (from 1.6 to 2.08 U/mL) produced a 3.6-fold increase in FAN yield. Maximum proteolytic conversion degree was 11.09 % (w/w) associated to a FAN production of 658 mg/L (Figure 6). Glucose and FAN concentrations achieved in this study are in the range of fermentation viability for yeast fermentation, as reported by Tsakona et al[19]. IP plays a key role in the conversion of sugar into intracellular lipids during fermentation[32] and therefore IP concentration was also monitored in this set of experiments. Under optimal fermentation conditions, as shown in Figure 6, IP concentration was higher than 1000 mg/L.

The last set of hydrolysis experiments was performed with 200 g/L of FW, 1.24 and 2.08 U/mL of glucoamylase and protease initial activities, respectively, at 55ºC for 60 h. Maximum FAN, glucose and IP values were achieved between 40 and 50 h of hydrolysis, with values of 650 mg/L, 96 g/L and 1200 mg/L, respectively. Nevertheless, nutrient production was nearly stable after 30 h, and optimal time of hydrolysis may be considered as 35 h, aiming to reduce process cost. Optimal hydrolysis conditions can be considered 200 g/L of FW, with initial enzymatic concentrations of glucoamylase and protease of 1.24 and 2 U/mL, respectively, at 55ºC for 35 h.

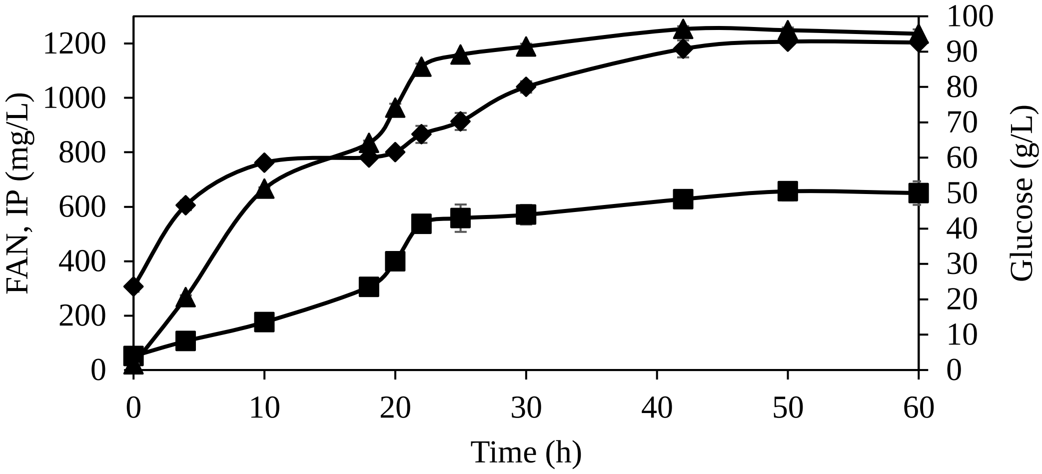


Figure 5-5 Glucose (▲), free amino nitrogen (FAN) (■) and inorganic phosphorous (IP) (●) production during food waste (FW) hydrolysis at 1.24 U/mL of glucoamylase activity and 2.08 U/mL of proteolytic activity, at 55°C and 200 g/L of FW

5.3.4 . Shake flask cultures of *R. toruloides* for microbial oil

The suitability of FWH as nutrient rich-medium for *R. toruloides* MO biodiesel production was evaluated in a set of flask experiments with 80 g/L initial glucose concentration at varying nitrogen concentrations (C/FAN ratios of 60, 120 and 180 w/w). Results, summarized in Table 3, show the highest productivity (0.03 g/Lh) for a C/FAN ratio of 180 w/w, attaining a TDW of 26 g/L, a lipid production of 6.2 g/L and 23.2% w/w of lipid content. Nevertheless, the highest biomass production (TDW 30.4 g/L) was detected for a C/FAN ratio of 60 w/w, at the expense of a reduction in lipid production (3.72 g/L) and lipid content (14% w/w). Results corroborate nitrogen-limited condition for lipogenesis in oleaginous yeast, thus doubling FAN concentration results in a reduction of biomass production and an increase in lipid production of 1.6 times. All fermentations were carried out over more than 200 h, but in the case of the fermentation with 180 w/w C/FAN ratio, time was reduced by 24 h, thus fermentation was completed after 216 h.

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Table 5-3 Shake flask cultures for *R. toruloides* fermentation in different C/FAN ratio at 30ºC.

C/FAN (w/w)	Time (h)	Sugar_0 (g/L)	Sugar_f (g/L^{-1})	FAN_0 (mg/L^{-})	FAN_f (mg/L^{-})	TDW (g/L)	Lipids (g/L)	Lipid content (%)	Productivity (g/Lh)
60	240	82.7±0.1	10.9±0.1	453±2	31±1	30.4±0.	3.72±0.04	14.01	0.02
120	240	80.4±0.1	7.3±0.3	274±2	33±1	26.5±0.5	4.47±0.07	14.45	0.02
180	216	82.7±0.9	7.1±0.1	213±8	26±2	26.3±0.1	6.2±0.1	23. 52	0.03

Sugar_0: initial sugar concentration; Sugar_f: final sugar concentration; FAN_0: initial free amino nitrogen; FAN_f: final free amino nitrogen; TDW: total dry wight

A second set of experiments (using a C/FAN ratio of 180 w/w) was performed to evaluate the role of trace element solution (TES) addition in shake flask cultures. Wang et al[12] reported a positive effect in *R. toruloides* in the presence of bivalent metal ions (*e.g.* Mg^{+2}, Ca^{+2}, Mn^{+2}) and trivalent metal ions (*e.g.* Fe^{+3}), which are required as a cofactor in enzymes involved in the citrate pathway, i.e. adenosin triphosfhate citralyse.

Results presented in Figure 7 show the evolution of biomass concentration (TDW), lipid production and nutrient consumption over time. Compared to optimal previous fermentations without trace elements, TDW increased 1.3 times (34.2g/L), lipid production almost doubled (12 g/L), nutrient consumption increased as well as productivity, the latter increased 2.3 times (0.07g/Lh), while fermentation time was reduced by more than 40 h (lipid production started after 100 h of fermentation, versus previous 159 h).

Wang et al[33] used multi-omic analyses with the main purpose of examining how phosphorous conditions affect lipid production in *R. toruloides*. Studies showed that IP limitation leads to up-regulation of metabolism pathways (associated to H^+/Pi-symporter), RNA degradation and triacylglycerol biosynthesis. In the present study, as showed in Figure 7, initial IP concentration was 300 mg/L and its consumption rate was similar in both types of fermentations (with and without TES) and comparable to that of FAN.

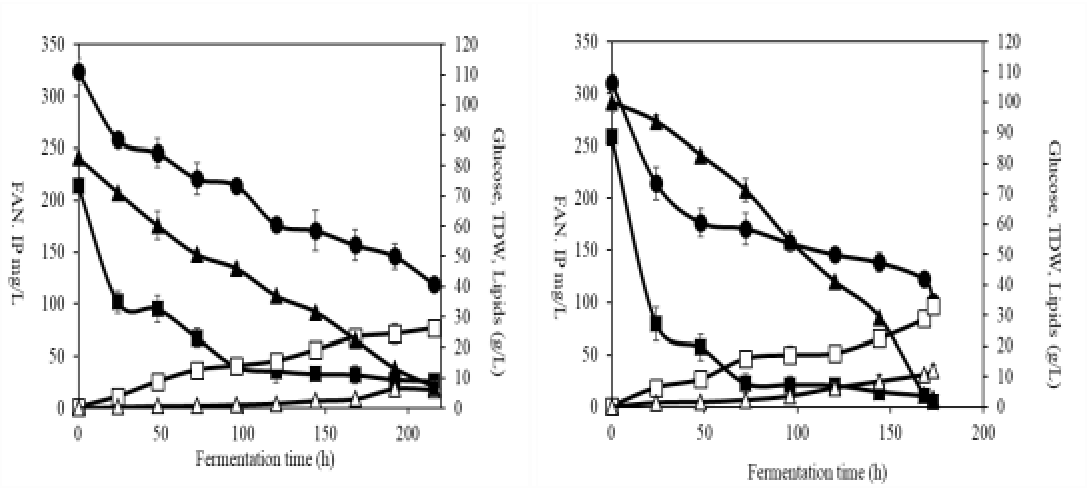

Figure 5-6. Consumption of glucose (▲), free amino nitrogen (FAN) (■) and inorganic phosphorous (IP) (●) and production of total dry weight (□)and microbial oil (△), using flask cultures of R. toruloides on filter-sterilized food waste hydrolysate (FWH) with (a) C/FAN ratio 180 w/w and (b) C/FAN 180 w/w ratio plus a trace element supplement

5.3.5 Feed-bath culture of *R. toruloides* on food waste hydrolysate

Synthesis of microbial lipids involves three different pathways (glycolysis, pentose phosphate and Kennedy) and three key molecules (acetyl-coA, citrate and malonyl-coA). Biochemical reactions are implicated in cytosol, mitochondria and endoplasmic reticulum of the cells. In this study, a feed-bath culture of *R.toruloides* was carried out under nitrogen limited conditions, using a glucose rich medium according to literature. Thus, oleaginous microorganism modifies its energetic balance due to nitrogen limitation. The adenosine monophosphate (AMP) concentration decreases and leads to an alteration of the krebs cycle and, as a consequence, iso-citric acid is accumulated in the cell mitochondria. This excess of citric acid leads to an exchange between citric acid (mitochondrial to cytosol) and malate (cytosol to mitochondrial). The citric excess in cytosol produces acetyl-coA by ATP-citrate lyase which are transformed in malonyl- Co A and produce fatty acids (FA). Finally, FA are transported to the endoplasmic reticulum, to be transformed into tryacylglicerides via the Kennedy pathway[16,34] . Feedback cultures leads to high cell density and high lipid production ratios. Fermentation condition was performed with the objective of producing the "de-novo lipid" to be transformed into biodiesel[35].

Two feed-batch experiments were carried out in a 3.5 L total volume bioreactor, using an FWH culture medium, supplemented with a solution of trace elements and IP. Initial glucose content was 60 g/L and two initial FAN concentrations were tested (800 mg/L –experiment a– and 400 mg/L –experiment b), under a pH of 6.

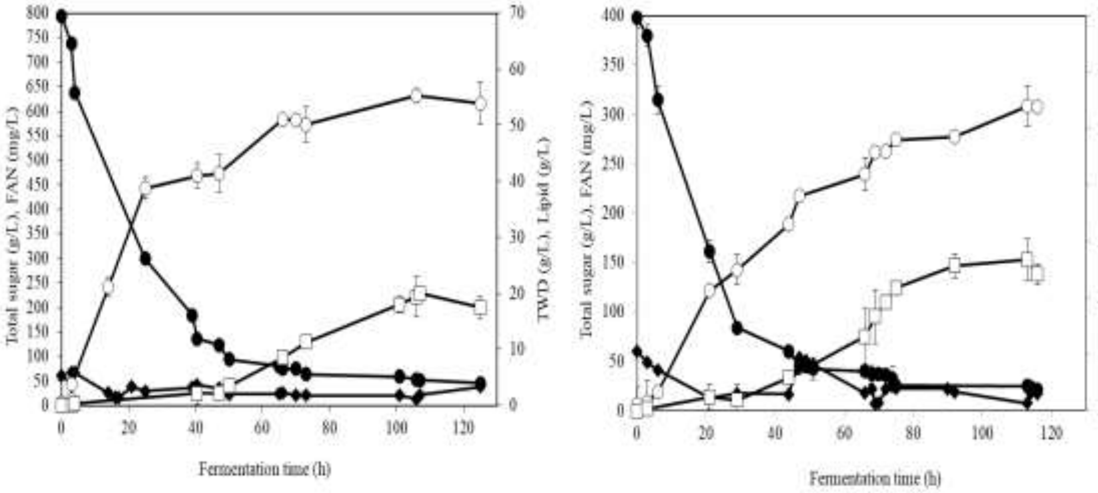

Figure 5-7. Consumption of glucose (■), free amino nitrogen (FAN) (●) and production of total dry weight (TDW) (○) and microbial oil (MO) (□), during feed-bath fermentation of *R. toruloides* on filter-sterilized food waste hydrolysate (FWH) with (a) initial FAN concentration of 793 mg/L and (b) initial FAN concentration of 397 mg/L, with 60 g/L of sugar and mineral supplement

Results of batch mode fermentations are showed in Figure 8 (a and b). No major differences were found for biomass production (TDW of 53.9 g/L and 53.8 g/L for experiments a and b, respectively). Lipid production started after 50 h of fermentation in experiment a (at 93.25 mg/L FAN), while in experiment b the initial lipid production started after 30-35 h (83.92 mg/L FAN). Values for maximum lipid production (Table 4) were 26.7 g/L (highest value), after 113 h of fermentation, with a productivity of 0.2 g/(Lh), achieved in experiment b, and 19.4 (g/L), after 107 h of fermentation, with a productivity of 0.1 g/(Lh) for experiment a. Results showed that under nitrogen limitation and depletion, lipid content was 1.5 times higher in experiment a (36.1 % w/w) than in b (52.8% w/w).

R. toruloides produces carotenoids as secondary metabolite, at the point between late log phase and early stationary phase[36]. Biochemical pathway is similar to that for lipids production: the presence of citrate in cytosol produces acetyl-co A that leads to ATP citrate lyase, which is then transformed by Acetyl-co acarboxylase into manolyl-Co (fatty acid synthesis) and also β-hydroxy β-methylglutaryl-CoA (HMG-CoA). This HMG-CoA is the precursor of mevalonic acid, that in humans leads to cholesterol biosynthesis and in yeast leads to carotenoids biosynthesis[16]. Carotenoid production in this study (Table 4) was optimal for experiment b, with 0.8 mg/L achieved, not as relevant as the value reported by Jaslyn et al.[36], that achieved 28.5 mg/L with *R. toruloides* ATCC 10788 in glycerol flask cultures. Nevertheless, the present study does not focus on carotenoid production and therefore further studies might be necessary to understand optimal conditions for carotenoid production in FWH cultures.

Table 4 shows lipid and carotenoid contents, as well as productivity of fermentations, reported in the literature, with different strains of *R. toruloides,* compared to the above mentioned results. Leiva-Candia et al.[9] evaluated *R. toruloides* DSM 444, using glycerol as carbon source with different nutrient supplements in a feed-batch bioreactor. Optimal results were obtained for sunflower hydrolysate, attaining 47.9 g/L of TDW and 18.1 g/L of lipids with a productivity of 0.14 g/Lh. In the same study, *R. toruloides* strain Y4 was also evaluated and reported for a biomass production of 31.1 g/L, a lipid production of 13.0 g/L and a productivity of 0.11 g/(Lh). Additional fermentation parameters have also been tested by other authors to improve productivity. As an example, Jingyang et al.[13] studied the use of surfactants in flask fermentations and obtained lipid concentrations of 7.1 g/L and lipid contents of 66% w/w. Another innovative study was the one developed by Zeng et al.[37] that mixed *R. toruloides* with oleaginous microalgae *Chlorella vulgaris* in a FWH flask culture, performing a lipid content of 25.5 g/L.

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Table 5-4. Fermentation productivity, microbial oil (MO) and carotenoid contents, of different *R. toruloides* strains from literature and present study

Strain	**time**	**Lipid production**	**Lipid content**	**Productivity**	**Carotenoid**	**Substrate**	**References**
R. toruloides	h	g/l	%	g/L h	mg/L		
Experiment a	107	19.4±0.5	36.1±0.5	0.18	0,8±0.1	FW hydrolysis	This study
Experiment b	116	26.7±0.4	52.9±0.2	0,2	0.7±0.1		
DSM 4444	192	n.d.	47.1	0.14	n.d.	Gly[1] +SFM hydrolysis	[12]
	166	n.d.	52.9	0.17	n.d.	Gly[1] +PSFM hydrolysis	
ATCC 10788	n.d.	n.d.	n.d.	n.d.	28.5	Glycerol (flask)	[39]
AS 2.1389	n.d.	n.d.	n.d.	n.d.	19.3		
CBS 5490		n.d.	n.d.	n.d.	18		
NCTC921	72	n.d.	32	n.d.	0.08	Glu[2]+ Nitrogen (flask)	[41]
21167	192	n.d.	63.4	n.d.	n.d.	Cassave starch Hydrolysis	[15]
AS 2.1389	136	n.d.	66	0.05	n.d.	Gly1+ yeast extract (flask)	[16]
Y4	n.d.	n.d.	41.8	0.2	n.d.	Gly[1] +Rapeseed hydrolysis	[12]

1: glycerol; 2: glucosa, ,n.d.: no data

5.3.6 Microbial oil profile

Microbial fatty acid profile, whose main fatty acids are C16 and C18, depends on synthesis pathways. In fact, profile depends on a sequence of repeated reactions catalyzed by a multi-enzyme complex called fatty acid synthase (FAS) which has two thiol groups (from Cys residue). One group of acetyl-co A and another of malonyl-co are condensed in four stages (condensation, reduction of the carbonyl group, dehydration and double bond reduction)[39]. Condensation cycle occurs seven times, leading to palmitate acid (C16:0) as a final product. Process continues, assisted by fatty acid elongase enzymes with specific and selective active site. Palmitate substrate is linked to two other carbon atoms from malonil-coA. Different elongase enzymes may catalyze fatty acid of C20, C22, etc[40].

MO fatty acid (FA) profile from this study, compared to profiles reported in the literature for different microbial strains of *R. toruloides*, is shown in Table 5. Main components of all reported MO are palmitoleic acid (C16:0) and oleic acid (C18:1), both added up to a value close to 75% of the total fatty acid content. Lower amounts of other fatty acids can also be found, such as stearic acid (C18:0) or linolenic acid (C18:2) with values below 9%; lauric acid (C12:0), not reported by other strains of *R. toruloides*, shows values slightly above 5% (w/w). A detailed review of Table 5 reveals several particularities, i.e. palmitoleic acid (C16:0) production by strain DMS444 is higher than that from other strains (above 33% w/w) or the different FA profile presented by NCT921 strain with a high concentration of stearic acid (C18:0). Moreover, Y4 strain has a high proportion of C18 fatty acids, with concentrations of stearic acid (18:0) above 10% (w/w) and linolenic acid (C18:2) of 19.2 % (w/w).

Table 5-5Fatty acid profile and total unsaturation degree of microbial oil produced by different *R. toruloides* strains and Common vegetable oils from literature

Fatty acid	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TUD[1]
Experiment a	5.1±0.2	1.3±0.4	25.3±0.1	1.7±0.1	8.8±0.4	49.5±0.1	5.8±0.3	0.2±0.2	0.6
Experiment b	5.3±0.3	1.8±0.1	26.3±0.1	1.7±0.1	8.8±0.3	50±0.8	5.4±0.1	0.3±0.6	0.6
DSM 4444[12]	n.d.	1.9	32.8	n.d.	8.9	48.6	5.7	n.d.	0.6
	n.d.	1.8	33.3	1.2	7.9	46.8	8.0	0.8	0.7
NCT921[41]	n.d.	0.1	6.8	0.0	71.1	6.5	2.7	0.4	0.1
21167[15]	n.d.	1.6	30.5	1.5	5.6	53.3	7.4	n.d.	0.7
AS 2.1389[39]	n.d.	1.8	27.4	1.6	11.9	47.4	7.8	n.d.	0.6
Y4[12]	n.d.	0.0	15.1	n.d.	12.0	53.7	19.2	n.d.	0.9
SFWO[27]	1.7	0.2	16.6	1.7	6.2	45.1	23.0	1.1	1.0
Sunflower[20]	n.d.	n.d.	6.1	0.1	3.7	22.7	65.0	0.8	1.6
Soybean[20]	n.d.	n.d.	11.5	2.4	2.6	19.4	47.4	16.9	1.7

1.Total unsaturation degree, TUD = (1 %MU + 2 %DU + 3 %TU)/100, where *%MU are mono-unsaturated methyl esters (%, w/w), %DU are di-unsaturated methyl esters (%, w/w) and %TU are tri-unsaturated methyl esters (%, w/w).*

According to Pinzi et al.[42], there is a correlation between unsaturation degree and biodiesel properties (i.e. cold weather behavior and oxidation stability), exhaust emissions and combustion quality. The presence of monounsaturated fatty acids has a positive effect on these properties. The balance between saturation and unsaturation in oleaginous yeast is controlled by a group of genes known as steoaroyl- Ca A desaturase (SCD). The double bond is catalyzed by Δ9-fatty acid desaturase (Δ9FAD), which adds a double bond between carbon 9 and 10 of palmitic and stearic acid[43]. The double bond on Δ12 carbon in stearic acid to form linolenic acid (C18:2) is correlated with fermentation time. Total unsaturation degree (TUD) for different strains is also shown in Table 5, where it can be appreciated that samples from this study show a TUD close to 0.6. All strains considered showed similar values with the exceptions of *R. toruloides* Y4, that presented a profile rich in oleic acid (C18:1) and linoleic acid (C18:2), and therefore a higher TUD value of 0.9; and *R. toruloides* NCT921, with a profile rich in stearic acid (C18:0), showing a lower TUD value. It can be assumed that, in general, TUD of MO from *R. toruloides* strains is lower than that of vegetable oils, namely sunflower or soybean.

To provide a better understanding of the relationships between FA profiles, a principal component analysis (PCA) of fatty acid profile from different origins was carried out and is shown in Figure 9. Fatty acid composition of the oil produced by different microorganisms, vegetables or animals, as well as waste oils, was reduced to two principal components, to make an easier pattern recognition. PCA explains more than 70% of the variability between fatty acid profiles. PC1 (OX axis) represents unsaturated profile distribution (C16:1, C18:1, C18:2 and C18:3) while PC2 (OY axis) represents saturated profile distribution (C14:0, C16:0 and C18:0). MO fatty acid profile may be classified into four groups, according to its composition. As shown in Figure 9, the red cluster is formed by vegetable oils (such as non-edible oil *Jatropha curcas*), and MO (*e.g.* from *Cryptococcus curvatus*) or SFWO. Main characteristic of this oil cluster is its high concentration of oleic acid (C18:1) followed by linoleic acid (C18:2).The green cluster is formed by vegetable oils, namely soybean, almond, etc., which are rich in unsaturated fatty acids but, in contrast with the red cluster, there is a high presence of linolenic acid (C18:3). The gray cluster is formed by oil produced by strains *Malus domestica, Candida* *pulcherrima* and others (Figure 9) and the main feature of this MO is the presence of palmitoleic (C16:1) and linoleic (C18:2) acids in similar concentrations. The black cluster is formed by oils with a high concentration of palmitic acid (C16:0) and it includes two strains of *R. toruloides*: As21389 and DSM 4444. The fatty acid profile of MO produced in this study shows a distribution close to green and red clusters, suggesting that FAME properties may be similar to soybean and SFWO. In next section, these aspects will be discussed in depth.

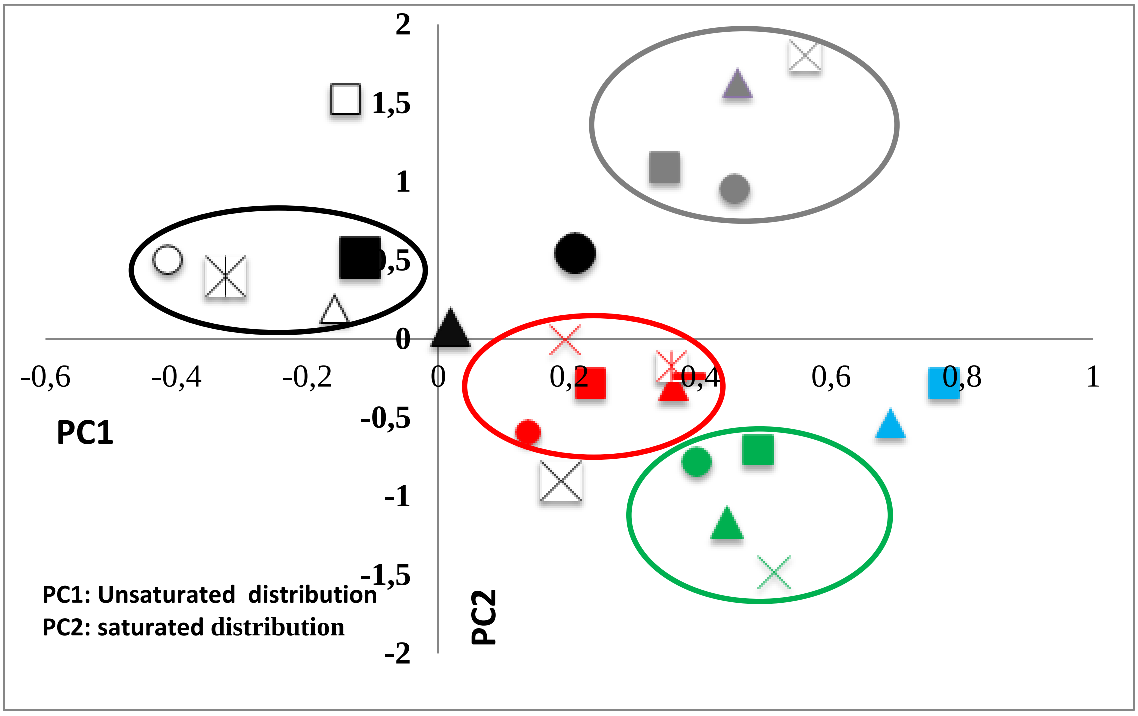

Figure 5-8. Correlation matrix between the principal component analysis (PCA) for different microorganisms Green cluster: Soybean[20] (▲), Almond[20] (■), Cottonseed[20] (●), Poppyseed[20] (X). Red cluster: SFWO[27] (▲), *Cryptococcus curvatus ATCC20509 [12]* (■) *Cryptococcus curvatus MUCL 29819[12]* (●), Jatropha curcas[20] (X), *Rhodotorula glutinis TISTR 5159 [12]* (-), *Yarrowia lipolytica* [13](*). *Grey cluster*: *Prunus domestica[14]* (▲), *Candida pulcherrima[14]* (■), Candida oleophila ATCC 20177 *[12]* (●), *Malus domestica[14]* (X). Black cluster: *Candida curvata NRRL-Y 151 [12]*(△), Candida boidinii ATCC 32195*[12]* (□), Brown Grease (○), R. toruloides AS 2.1389 (*), *R. toruloides DSM 444[12]* (■), others *R. toruloides Y4[12]* (▲), *R. toruloides 21167* [15] (●), *R. toruloides from* this study (X), yellow Grease [20] (▲), Rapeseed [20] (■).

Other factors with influence on MO FA profiles are fermentation conditions (*e.g.* pH or oxygen concentration) and substrate (type of carbon, trace element or nutrient limitations, etc.) which can affect cell homeostasis process. In this study (as shown in Table 5) there are no significant differences in fatty acid profile between experiments.

These results agree with Leiva et al.[9] that tested DMS444 strain with different nitrogen substrates and glycerol as a carbon source, and observed that there were no significant differences in fatty acid profiles (Table 5).

5.3.7 Fatty acid methyl esters properties

The use of biodiesel depends on its ability to reproduce diesel fuel behavior in an engine, but reducing exhaust gas emissions.European biodiesel standard EN 14214 provides parameter thresholds that must be met by FAME fuels. Table 6 shows biodiesel properties from the transesterified MO produced in this study. FAME conversion was 94.6 % (w/w), just two points below the threshold required by EN 14214. Transesterification reaction may be affected by several factors, for example, the presence of phospholipids may inhibit alkali catalysts during transesterification[44], which could explain the under normative yield obtained values. *R. toruloides* also present carotenoids (with concentrations in the range of 800 µg/g, Table 4) which have antioxidant properties and it is not clear yet whether they have a positive or negative influence on transesterification.

Table1-6. Fatty acid methyl ester (FAME) properties of transesterified oil from *R. toruloides*

FAME Properties		
QUALITY PARAMETER	**TEST METHODS**	**VALUES**
FAME (% w/w)	EN 14103; Min: 96.50	94.6 ± 0.6
Linolenic ester content (%, w/w)	EN 14103; Max: 12	0.2 ± 0
Kinematic viscosity at 40 ºC, µ (mm^2/s)	EN ISO 3104; 3.5-5.0	4.7 ± 0.4
Low calorific value (MJ/kg)	ASTM D240; Min: 35000	39.5 ± 0.1
Cold filter plugging point[1] (ºC)		3 ± 0.3
Water content (ppm)	EN ISO 12937; Max: 500	249 ± 48
Flash point (ºC)	EN ISO 3679; Min: 120ºC	158.8 ± 15.1
Carbon residue (% w/w.)	EN ISO 10370; Max: 0.30	0.15 ± 0.1
Oxidation stability at 110 ºC (h)	EN 14112; Min: 8	6.1 ± 0.1
Cetane number[2]	Min: 50	68.82
Acid value (mg KOH/g)	EN 14104; Max: 0.50	0.5 ± 0.1

1,2: Calculated according to Pinzi et al[20]

Lower calorific power (LCP), cetane number (CN), kinematic viscosity (μ) and carbon residue (CR) were evaluated to study the viability of this fuel for diesel engines. LCP indicates the amount of energy that could be transferred during the combustion and its value is directly related to fuel elemental composition (carbon, nitrogen and oxygen)[41]. Literature shows FAME LCP values varying between 36 to 41 MJ/Kg, while minimum value required by EN 14214 is 35 MJ/kg. Therefore, obtained LCP value of 38.5 MJ/kg agrees with both standard and literature. Cetane number allows an evaluation of the combustion quality during ignition, providing information about ignition time delay in the combustion chamber. Low CN values are related to an inefficient combustion process, thus there is an increase in gaseous and particle exhaust emissions[45]. In this study, CN was calculated using a mathematical prediction model based on TUD and length of chain (LC) calculated values[41], and had a value of 68.2, which is within the limits described by European normative. Kinematic viscosity plays an important role in fuel injection during combustion, thus high viscosities are responsible of variations in injection volume, reducing combustion efficiency[46]. In the present work, a ν of 4.7 mm^2/s was obtained, within standard limits. CR shows the presence of other residues in biodiesel. In this study, CR was reported as 0.15% (w/w), lower than standard threshold, even though it might be expected that the presence of carotenoids may increase CR value[47].

Storage, related to FAME stability, is one of the most important aspects of biodiesel production. During manufacturing process, MO is exposed to high temperatures and solvents that can produce a chemical breakdown. Moreover, combination of water, free fatty acids and linolenic acid may contribute to generate side reactions which can reduce storage time and produce engine damage[41]. First step after MO transesterification consists in a washing treatment to remove the polar fraction. Water is the most popular solvent used in this step; water content value obtained in FAME samples was 249 ppm, below standard value (500 ppm). Free fatty acid content was 0.5% (w/w), a value within the limit established by EN 14214. Linolenic methyl ester was also checked due to reactivity of triple bond, that may decrease FAME oxidation stability, although, in this case, the value agreed with European standard. Oxidation stability is key property to evaluate the storage behavior. This property depends (apart from all parameters mentioned above) on FAME unsaturation degree and other components present in the biodiesel that may originate side reactions. In this study, the value obtained of 6.1 h was below EN 14214 regulation (8 h)[41]. To improve this property, and meet required threshold, antioxidants (*e.g.* pyrogallol (PY), tertiary-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT)) in concentrations between 1,000 ppm and 8,000 ppm may be added[48]. However, *R. toruloides* produces carotenoids with antioxidant properties, and therefore, to study culture conditions to increase carotenoids concentration could be an option.

CFPP gives information about fuel behavior under cold climate conditions, thus low values may lead to potential filter obstruction and subsequent compression-ignition engine problems. There is no specific recommendation in the European standard based on regional climate. In this study, the value was 4.5ºC, which may be improved by blending biodiesel with diesel fuel or bioalcohols, i.e. butanol or methanol[49]. Finally, FP, a safety parameter with a required minimum value of 120ºC after EN 14214 normative, was analyzed and reported as 158ºC. In general, obtained FAME from *R. toruloides* MO has shown viable properties to be used in diesel engines when additived to improve its oxidation stability.

Conclusions

Results show that a sustainable biorefinery, based on the valorization of food-industry wastes using *Rhodosporidium toruloides Y-27012*, for the production of microbial oil that is transesterified into biodiesel, is feasible and it is in accordance with European biodiesel standard EN 14214. Suitability of food waste to produce an alternative culture medium for microbial oil production in two steps (solid state fermentation and hydrolysis) has been demonstrated. *A. awamori* provides an appropriate crude enzyme consortia, able to degrade starch and protein into adequate nutrients for *Rhodosporidium toruloides Y-27012* fermentation. Produced microbial oil presented similar fatty acid profile than other raw materials, i.e. poppy-seed, solid food waste oil or *Cryptococcus curvatus*. Biodiesel showed properties, most of them within the limits described by European normative.

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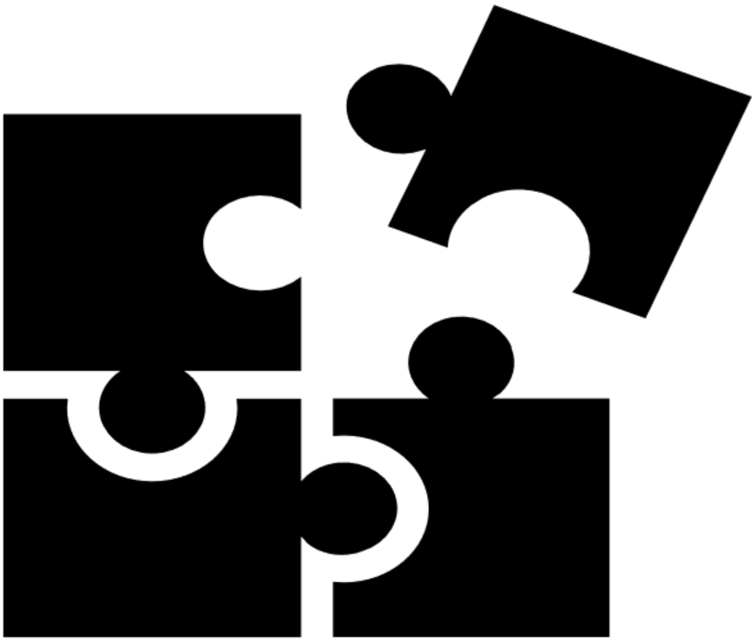
CHAPTER 6.

CONCLUSIONS AND FUTURE RESEARCH LINES



6.1. Conclusions

Food waste typology analysis showed a high presence of rich-in-starch foodstuff. The chemical quantitative analysis showed a high concentration of lipids, starch and protein and presented a profile with a high degradation potential, which can be used for direct and indirect valorization through biorefinery processes. Statistical analysis helps to understand the complex matrix of FW and shows the importance of developing a specific analysis.

The lipid fraction extracted from Solid Food Waste (SFW) from different restaurants showed some significant differences in fatty acid composition. However, this composition was similar to that of vegetable oils. In spite of these differences, physical and chemical properties were similar between oils, with the exception of acid value. Consequently, it seems possible to work with mixtures of oils extracted from SFW from different restaurants to produce biodiesel. Thus, as these oils show a strong tendency to present a high content of free fatty acids, a preliminary acid value analysis and a further acid catalyzed-esterification pre-treatment are needed. Compared with conventional methods, US-assisted reaction provided significant advantages in terms of reaction time and energy savings. Physical and chemical property analysis showed that biodiesel fulfils European biodiesel standards, except regarding oxidation stability, FAME yield and glyceride content. For this reason, this biofuel can be used as fuel by either blending it with diesel fuel or adding phenolic antioxidants.

Finally, results show that a sustainable biorefinery, based on the valorization of food-industry wastes using *Rhodosporidium toruloides* Y-27012, for the production of microbial oil (MO) that is transesterified into biodiesel, is feasible and in accordance with circular economy principles. The suitability of food waste to produce an alternative culture medium for MO production in two steps (solid state fermentation and subsequent hydrolysis) has been demonstrated. *A. awamori* presented a perfect crude enzyme consortium, able to degrade starch and protein into adequate nutrients for *Rhodosporidium toruloides* Y-27012 fermentation. MO profile presented a similar fatty acid profile to that of other raw materials, such as poppy-seeds, solid food waste oil or *Cryptococcus curvatus*. Biodiesel showed properties within the limits described by the European biodiesel standard EN 14214.

6.2. Future lines

Given the results provided previously, any future work should focus on four different areas.

1. Chemical and physical analysis, combined with statistical tests, provide a good starting point for the basis of a FW biorefinery. However, FW analysis must be improved due to its complex chemical matrix. New instrumental techniques should be tested, such as near-infrared spectrometry and new methodologies of analysis should be incorporated, i.e. chemometric tools, which would allow the use of an extensive data set, while developing prediction algorithms. These algorithms may be incorporated in biorefinery life cycle assessment, thus leading to an improvement in productivity and increasing economic yields.
2. Several chemical and physical properties of SFW oil biodiesel were analyzed. It was observed that only oxidation stability, FAME yield and glyceride content did not fulfil European standard EN 14214. For this reason, new lines of work to improve biodiesel quality should be focused on a further reaction optimization study. Also, blending biodiesel with either diesel fuel or additives is strongly recommended and to corroborate this statement, further diesel engine tests are needed.
3. Improving MO production methodology. Improving fermentation medium, with the use of different microorganisms and consortia for better performance in nitrogen processing; thus, lignocellulosic fiber could be processed. Also, improving fermentation conditions with different C/N ratios, that should result in an increase in biomass and MO production. Also, including new kinds of energy, e.g. ultrasound for MO extraction, which may reduce energy consumption, organic solvents and also the number of steps in the process, due to being able to perform extraction and transesterification simultaneously.
4. Developing new high value-added products, e.g. both the lipid fraction from solid food waste and MO could be used to produce bio-lubricants. This methodology may involve the use of US and enzymatic catalysis, with the objective of reducing energy consumption and the request of chemical compounds. Bio-oil may be produced using the lignocellulosic material that cannot be used in hydrolysis. For this process, glycerol from biodiesel production could be used as a solvent, while energy consumption may be reduced using US.

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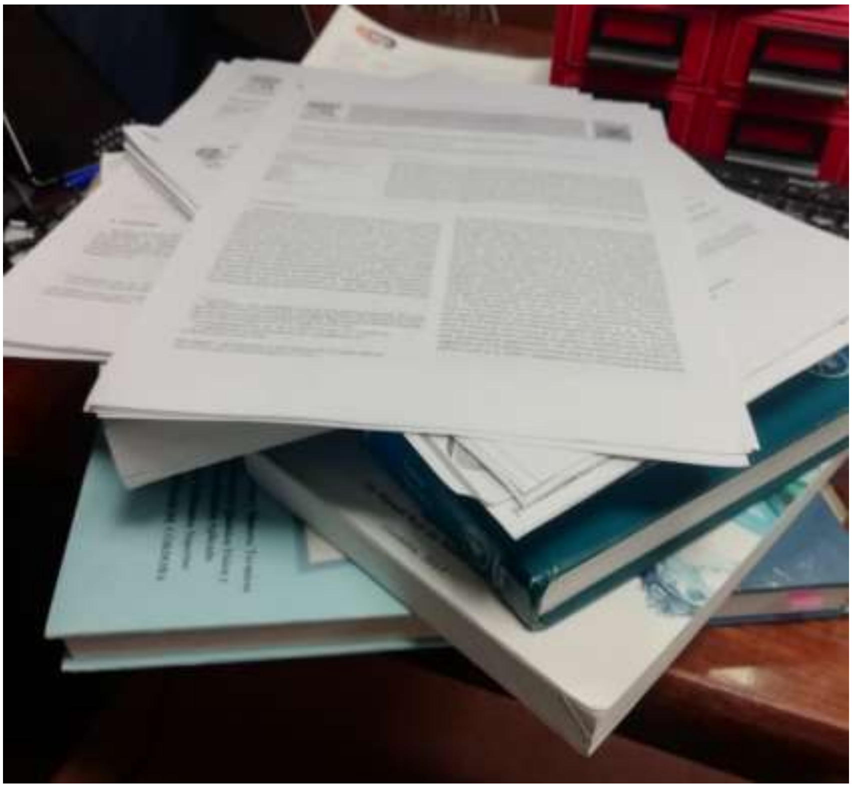

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APPENDIXES

Appendix 1

Journal: Current Opinion in Green and Sustainable Chemistry
ISSN: 00162361

Volume 14, December 2018, Pages 67-79

Link

https://doi.org/10.1016/j.cogsc.2018.06.011

Tittle
Valorization of food waste based on its composition through the concept of biorefinery
Authors
Miguel Carmona-Cabello, Isabel L. Garcia, David Leiva-Candia, M. Pilar Dorado

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in
Green and Sustainable Chemistry

Valorization of food waste based on its composition through the concept of biorefinery

Miguel Carmona-Cabello, Isabel L. Garcia,
David Leiva-Candia and M. Pilar Dorado

Waste management is one of the most significant challenges of EU policies for the coming decades. Our changing food habits carries an inherent increase in waste nutrients, that worth recycling. The traditional method of waste management is not adapted to meet future energy and environmental requirements. Food waste (FW)-based biorefinery allows new approaches in waste recovery, while improving industrial processes. However, interactions between FW nutrients, their derivates and subsequent treatment methodologies may lead to negative effects and bioprocess low yield. This work aims to state the importance of new strategies in biorefineries, based on FW nutrients and their interactions.

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Current Opinion in Green and Sustainable Chemistry 2018, **14**:67–79

This review comes from a themed issue on **Biofuels and bioenergy 2018**

Edited by **Carol Sze Ki Lin** and **Vasiliki Kachrimanidou**

Available online 27 June 2018

https://doi.org/10.1016/j.cogsc.2018.06.011

Introduction

Food waste (FW) is defined as either edible or non-edible food that is discarded in any step of the food chain: primary sector manufacturing steps, logistics (food damaged during transportation), storing operations (expiration date label, quality losses) and post-consumer steps [1]. FW from primary sector (also known as pre-consumer step) is composed of food that do not reach consumer because it is either discarded or recycled. Non-edible food pieces, food that does not comply with organoleptic standards, food from process failures or batches with microbial contamination are usually discharged during manufacturing step. Last chain step, known as post-consumer step, includes residues from household and food service sector (mainly restaurants and hospitals) and are basically composed of peels, bones, cooking oil, discharged food portions, etc. FW is generally considered non-hazardous waste, with the exception of animal waste that is controlled by European regulation (EC) No 1069/2009. Just in 2014, 2503 million t of waste [2], derived from household and economic activities, were generated in the EU-28, which means an average per inhabitant of 4.9 t/year. From that figure, *ca.* 10% corresponds to municipal solid waste (MSW), which includes around 45–55% of FW [3]. It is worth to mention that MSW control is a crucial target for EU environmental policy.

In the past 10 years, EU waste management has dramatically changed. In 2005, 63% of MSW were disposed in landfills, while ten years later, this percentage has been reduced to 24%. Moreover, in 2005, only 11% of MSW was recycled, while ten years later it increased above 25%. EU goal is to reach 60% recycling share by 2025 [2].

In terms of energy efficiency, a waste is suitable for incineration if the lower heating value is above 3344 kJ/kg [4]. Due to FW high moisture content, lower heating value is reduced. In any case, direct energy recovery through incineration provides values in a range from 3599.21 kJ/kg to 4956.77 kJ/kg, above the threshold. However, green-house gas emissions (GHG) increase [5]. An environmentally friendly recovery approach is provided by the biorefinery philosophy [6–8]. Through the refinery concept, a biorefinery fits the processing based on the raw material, with the target of maximizing both recovered nutrients and production of high value-added products. A biomass-based biorefinery may produce fuels, electricity, combined heat and power and several products (i.e. plastics), analogously to petroleum refinery, maximizing the value derived from biomass components and intermediates. Biomass-based biorefinery helps to reduce energy costs and GHG, compared to traditional energy generation plants. Biorefineries are still under research, but they are expected to play a significant role in the future of energy, material and chemical generation. Due to its properties, FW is a solid candidate as biorefinery raw material.

Hence, the objective of this work is to discuss the composition of FW and analyze its role in biorefineries, finding out nutrient synergies and their applications within biorefinery processes.

Appendix 2

Journal: Fuel

ISSN: 00162361

Link

https://doi.org/10.1016/j.fuel.2017.11.096

Volume 215, 1 March 2018, Pages 492-498

Tittle

Valorization of food waste from restaurants by transesterification of the lipid fraction(Article)

Authors

Carmona-Cabello, M., Leiva-Candia, D., Castro-Cantarero, J.L., Pinzi, S., Dorado, M.P

Fuel 215 (2018) 492–498

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

Valorization of food waste from restaurants by transesterification of the lipid fraction

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ARTICLE INFO

Keywords:
Biodiesel
European standard
Quality
Biofuel
Biorefinery

ABSTRACT

Food waste contributes to increase the environmental impact, besides the ethical issue. One interesting way of valorization is its conversion in biofuel, thus helping to boost the concept of circular economy. The target of this work was to find out the feasibility of the use of the oil included in solid food waste (SFW) to produce biodiesel that meets the European biodiesel standard EN 14214. For this purpose, Soxhlet extraction of the lipid fraction of SFW from different restaurants has been carried out. Fatty acid composition was analyzed and potential differences concerning the source of SFW were evaluated through principal component analysis. Results showed significant differences in the oil fatty acid composition depending on the restaurant. However, oil physical and chemical properties were similar, excepting the acid value. Due to the high free fatty acid content (acidity of 11.21 mg KOH/g) of the oil from fine dining restaurant residues, acid-catalyzed esterification pre-treatment to the alkaline transesterification was needed. The fatty acid composition of oils from SFW differs depending on the restaurant, but the range of fatty acid methyl esters (FAME) is similar to that found in vegetable oils, showing a content of oleic acid (C18:1) between 36.39 and 41.57% w/w and linolenic acid (C18:2) of 21.37–38.63% w/w. Several chemical and physical properties of SFW oil biodiesel were analyzed. It was found that biodiesel fulfil the European standard EN 14214, with the exception of FAME yield, oxidation stability and glyceride content. For this reason and to improve biodiesel quality, further reaction optimization study, blending with diesel fuel or the use of additives is strongly recommended. It may be concluded, from this field trial, that oil from SFW from different restaurants may be mixed together and used to produce biodiesel. To corroborate this statement, further diesel engine tests are needed.

1. Introduction

Solid food waste (SFW) comprises food residues and is composed of processed food or discarded edible raw materials [1]. Recently, FAO has reported that more than 50% of the food produced is discarded, reaching over 1.3 billion tons of wasted food per year [2]. EUROSTAT data from 2006 show an annual food waste generation in EU27 of 89 Mt, equivalent to 179 kg per capita. Considering EU population growth by 2020, a production of SFW by about 126 Mt is expected, corresponding to an annual CO_2 related emissions of 240 Mt [1]. These figures demonstrate that SFW has a great impact on environment, food quality, safety and security; a sustainable management of SFW represents a challenge from an economic and ecological point of view. Furthermore, food waste involves a relevant ethical issue, as while food is wasted by developed countries, one billion people die of starvation in the rest of the world [3]. Different sources of SFW production have been identified [1]:

- Manufacturing sector
- Household sector
- Wholesale/retail sector
- Food service sector

In the EU27, 14% of total SFW is provided by the food service sector, reaching 12.3 Mt (an average of 25 kg per capita). Also, there is a significant discrepancy when either EU12 or EU15 is considered, as SFW from food service sector provides 12 kg or 28 kg SFW per capita, respectively. SFW production from food services is caused by inadequate storage, technical malfunction while food is processed in the food chain supply, food safety regulations or excess of food vs. request

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https://doi.org/10.1016/j.fuel.2017.11.096
Received 23 September 2017; Received in revised form 22 November 2017; Accepted 23 November 2017

Appendix 3

Journal: Fuel

ISSN: 00162361

Volume 255, 1 November 2019, 115817

Link

https://doi.org/10.1016/j.fuel.2019.115817

Tittle

Optimization of solid food waste oil biodiesel by ultrasound-assisted transesterification.

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Carmona-Cabello, M., Sáez-Bastante, J., Pinzi, S., Dorado, M.P

Fuel 255 (2019) 115817

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

ELSEVIER

Full Length Article

Optimization of solid food waste oil biodiesel by ultrasound-assisted transesterification

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A R T I C L E I N F O

Keywords:
Biofuel
Food waste
FAME
Biorefinery
Auxiliary energy

A B S T R A C T

Substitution of fossil diesel fuel by biodiesel is a realistic alternative due to its compatibility with commercial diesel engines. However, detractors consider that traditional raw materials used for biodiesel production may compete with human feeding (edible vegetables oils), land and water. So, new raw materials are needed. Annually, around 1.3 billion ton of food are discarded or wasted. This huge figure has led researchers to seek for new applications to this carbon source. The main goal of this work is biodiesel production optimization (with and without auxiliary energy assistance), and further characterization, by using the lipid fraction included in solid food waste, collected from local restaurants. Solid food waste oil (SFWO) characterization showed a fatty acid profile rich in oleic (C18:1), palmitic (C16:0) and linolenic (C18:2) acids, a remarkable fatty acid composition suitable for biodiesel production. Due to high free fatty acid content, acid esterification pre-treatment was conducted, followed by a basic transesterification optimization, by both conventional and ultrasound (US) assisted reactions. Response surface methodology was selected to perform the experimental design; optimal conditions for conventional transesterification were achieved at 6.08:1 methanol-to-oil molar ratio, 1.28% w/w catalyst and 52.5 °C reaction temperature, providing 93.23% w/w fatty acid methyl esters (FAME) conversion. Biodiesel quality was also analyzed with excellent results considering cold properties (cold filter plugging point below −3 °C), although oxidation stability did not fulfill European standard limit. US-assisted reaction was also compared with conventional transesterification in terms of energy consumption and reaction time, providing significant savings in both energy and reaction time (40 min reduction). As a conclusion, US-assisted SFWO biodiesel meets most European standard limits, following circular economy. To extend its use, blends with diesel fuel and antioxidant addition are recommended, besides long-term engine tests.

1. Introduction

Over the next 50 years, human society must find solutions to multiple challenges due to exponential population growth. One of the most important issues to be faced derives from the use of fossil fuels. Oil price increase (due to fluctuations in the markets), environmental and health deterioration, besides geopolitical instability, are taking special relevance and require short-term resolution.

For the reasons above, a growing interest in searching for new biofuels as a replacement of fossil fuels that are compatible with internal combustion engines has been promoted. Biofuels are becoming a new alternative to reduce both dependency on fossil fuels and greenhouse gas emissions. Biofuel sector produces 133 billion liters per year and employs a total of 1 678 000 people [1]. In this industry, bioethanol and biodiesel are the worlds most abundant biofuels. Most biodiesel production is used in the transport sector. Considering last 15 years, its production has grown by 6.5%. Nevertheless, despite the tendency of growth in the previous years, biodiesel production in 2015 was 30.1 billion litres, which represents one per cent of decrease in growth with respect to 2014. It may be due to an increase in raw material prices and restrictive policies in the use of edible crops [1]. Furthermore, 95% of biodiesel feedstocks come from edible crops such as palm, soybean and rapeseed oils [2]. According to the literature, intensive soil use could negatively affect the food supply chain and environmental equilibrium [3]. This fact has led to search for alternatives to these edible crops, being the use of non-edible ones among the first evaluated options. In this way, species like *Jatropha curcas*, *Camelina sativa* or *Sinapis alba* have been tested with interesting results.

Given the controversy regarding biofuel production, both industry sector and scientific community are evaluating new feedstocks. In this

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https://doi.org/10.1016/j.fuel.2019.115817
Received 15 May 2019; Received in revised form 12 July 2019; Accepted 15 July 2019
0016-2361/

Appendix 4

Journal: Industrial Crops and Products

ISSN: 09266690

Volume 139, 1 November 2019, Article number 111535

Link

https://doi.org/10.1016/j.indcrop.2019.111535

Tittle

Biodiesel production from microbial oil provided by oleaginous yeasts from olive oil mill wastewater growing on industrial glycerol

Authors

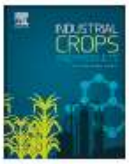
Chebbi, H., Leiva-Candia, D., Carmona-Cabello, M., Jaouani, A., Dorado, M.P.

Industrial Crops & Products 139 (2019) 111535

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Biodiesel production from microbial oil provided by oleaginous yeasts from olive oil mill wastewater growing on industrial glycerol

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ARTICLE INFO

Keywords:
Oleaginous yeasts
Microbial oil
Biodiesel standard
Fed-batch
Fatty acid methyl ester

ABSTRACT

This study focuses on the potential of four lipogenic yeasts to produce lipids using glycerol as carbon substrate; *Candida molendinolei* MH267796, *Meyerozyma caribbica* MH267795 *and Rhodotorula minuta* MH267794 were isolated from olive oil mill wastewater, while *Cryptococcus curvatus* ATCC20509 was used as control strain. Lipid production with crude glycerol was higher than that provided with pure glycerol. Fed-batch bioreactor cultures with *M. caribbica* MH267795 led to the production of 42.12 $g L^{-1}$ of total dry weight with a microbial oil content of 48.14% ($w w^{-1}$). In general terms, tested biodiesel properties meet all the limits set by European biodiesel standard UNE 14214:2013, excepting fatty acid methyl ester content. In addition, *M. caribbica* MH267795 oil biodiesel depicts a fatty acid methyl ester content (90.2% ($w w^{-1}$)) higher than that of the most intensively studied *C. curvatus* oil biodiesel. Re-use of crude glycerol as raw material in biodiesel industry has been proven through fermentation, increasing potential full-scale application of such alternative.

1. Introduction

Biodiesel is considered an attractive alternative to fossil-based diesel fuel because of its environmental benefits. It is produced from biomass by transesterification of triacylglycerols, yielding monoalkyl esters of long-chain fatty acids with short-chain alcohols, for example, fatty acid methyl esters and fatty acid ethyl esters (Meng et al., 2009). In different parts of the world, various renewable lipids have been chosen for the production of biodiesel, including mainly vegetable oils, in addition to animal fats and waste oils (Manaf et al., 2018). However, the use of vegetable oils as raw material for biodiesel production could compete with their use as edible oils, thus potentially leading to soaring food prices and limiting the large-scale development. Recently, much attention has been paid to the development of microbial oils and it has been found that many microorganisms, i.e. algae, bacteria and fungi, have the ability to accumulate oils similarly to vegetable oils (Liu et al., 2011) under appropriate cultivation conditions. Microorganisms that can accumulate above 20% of their biomass in lipid form are considered as oleaginous species. However, microbial lipids high processing cost may limit full scale application. Thus, searching for high lipid producing microbes, using low cost raw materials and improving production process would allow the reduction of overall production cost. In this regard, cells of oleaginous yeasts (OY) accumulate high quantities of lipids when cultivated in media with excess quantities of carbon, such as sugar, polysaccharide, or glycerol (a by-product from biodiesel industry), under nitrogen starvation. Currently, only 3–10% of the 1600 known yeast have been reported as oleaginous yeast species (Sitepu et al., 2014). These included various species of the phylum Basidiomycota, e.g. *Cryptococcus curvatus, Cryptococcus laurentii, Cryptococcus podzolicus, Cryptococcus terricola, Rhodosporidium fluviale, Rhodosporidium rubra, Rhodosporidium toruloides, Rhodotorula glutinis* and *Sporidiobolus ruineniae*, and the Phylum Ascomycota, e.g. *Candida tropicalis, Candida utilis, Kodamaea ohmeri, Lipomyces starkeyi, Lipomyces lipofer* and *Yarrowia lipolytica* (Castanha et al., 2014; Kanti et al., 2013; Kitcha and Cheirsilp, 2013; Meesters et al., 1996; Pan et al., 2009; Papanikolaou and Aggelis, 2011; Ratledge and Cohen, 2008; Schulze et al., 2014; Sitepu et al., 2012). Yeasts exhibit advantages for lipid production over other microbial sources, namely, show fast growth

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https://doi.org/10.1016/j.indcrop.2019.111535
Received 16 October 2018; Received in revised form 26 June 2019; Accepted 2 July 2019

Appendix 5

Tittle

The concept of biorefinery: valorization of food waste from restaurant

Special mention

Poster award breakthrough initiative

Author

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THE CONCEPT OF BIOREFINERIES:
VALORISATION OF FOOD WASTE FROM RESTAURANTS

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Albi (France)
May 25-28, 2014

Introduction

Food waste (FW) and by-product streams are generated along any supply chain of industrial food and household processing. Considering that one-third of the food intended for human consumption is wasted worldwide, problems derived from economic losses and environmental pollution may arise (Gustavsson el al., 2010). This study proposes a biorefinery concept through valorization of FW from restaurants. The chemical characterisation of FW was shown high content of starch, protein and lipids. Enzymatic hydrolysis of FW using crude enzyme consortia, produced by *Aspergillus awamori*, was performed to produce a generic fermentation media, thus assessing its potential as feedstock for microbial oil, which will further serve as raw material for biodiesel production. Also, the oil extracted from FW was studied and found that meet the requirements of UNE EN ISO for biodiesel properties.

Materials & Methods

Raw materials: FW was collected from four different restaurants in Cordoba (Spain): campus restaurant, Italian restaurant, hotel restaurant and meat grill.

Microorganism: *A. awamori* 2B.361 U 2/1 was kindly provided by Professor Colin Webb (University of Manchester, UK) (Wang et al., 2009).

Collection and pretreatment of FW: With the aim to evaluate the homogeneity, FW was collected daily during a 7-day period of time and later once per week during two months. Samples were lyophilized and grinded to fine powder.

Solid state fermentation (SSF): crude enzymes production by *A. awamori* was carried out in 250 mL flasks containing 5 g of dried potato peel (PP) after incubation at 30 °C/ 48 h. PP composition was 38 % (w/w, db) starch, 66 % (w/w, db) NDF and 3% (w/w, db) protein content.

Enzymatic hydrolysis of FW: Fermented solids from SSF were macerated and suspended in sterilized tap water. Hydrolysates were produced by mixing varying quantities of FW streams with the enzyme-rich suspension in 1 L-Duran bottles (Tsakona et al., 2014).

Biodiesel production: The oil extracted from FW was transesterified into biodiesel. When the oil acid value was >3, a previous esterification step was needed (8:1 ratio of methanol:oil, 1 % (w/w) of sulfuric acid as catalyst, conducted at 72 °C/ 2 h, followed by transterification. In case the acid value was <3, a direct transesterification with a methanol:oil ratio of 6:1 and potassium hydroxide 1.5 % (w/w) as catalyst was used. Biodiesel properties were also studied (Pinzi et al., 2009).

Analytical methods: One-way ANOVA and TUKEY was used to asses the significance of the daily and weekly differences between restaurants. The significance was determined with P-values, $p < 0.05$ corresponding to 95% confidence level.

Sample composition analysis was conducted in the Agrifood laboratory of Cordoba (*Junta de Andalucía*). Biodiesel composition, yield and properties were analysed according to EN 14214 standard.

Results & Discussion

Characterization of FW

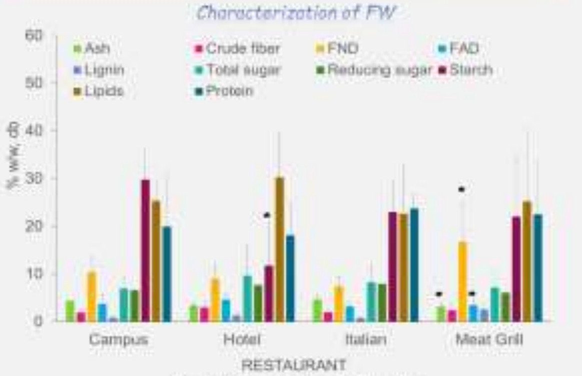


Figure 1. Composition of food waste

- Starch (22% w/w, db), protein (22% w/w, db) and lipid (26% w/w, db) were the major components. Main micronutrients in FW were metals, namely Fe, Mn, K, Na, Zn and very low levels (< 0.06 ppm) of Pb and As.
- Differences in ash ($p = 0.01$), neutral detergent fiber (NDF) ($p = 0.03$) and lignin ($p = 0.0042$) provided by the meat grill were significant, whereas regarding the hotel restaurant, differences in starch ($p = 0.0042$) were significant, the rest of components showing $p > 0{,}05$.

Oil extraction from FW

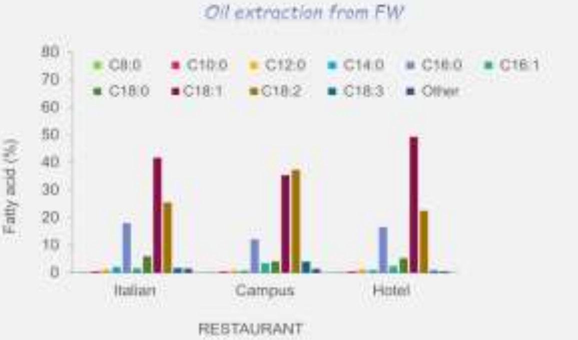


Figure 2. Composition of fatty acid from FW oil-derived biodiesel

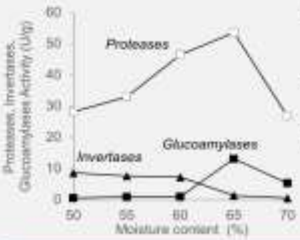
- The fatty acid profile was consisted mainly of palmitic (C16:0), oleic (C18:1) and linolenic (C18:2) acid.
- Statistical analysis found differences between FA profiles of biodiesel depending on the restaurant.

Biodiesel properties

Table 1. Physical and chemical properties of FW oil-derived biodiesel

Restaurant	Yield	Carbon residue	Estability oxidation	Cetane number	Flash point	Viscosity
	(%)	(% w/w)	(h)		(°C)	(mm²/s)
Italian	89.44	0.039	3.91	58.75	161.29	5.07
Campus	84.17	0.038	1.43	53.56	155.20	4.70
Hotel	84.58	0.046	1.94	58.24	164.50	5.12
UNE EN ISO	> 96 (14103)	< 0.03 (10370)	> 6 (14107)	>51 (5165)	122 (14214)	3.5-5.5 (3104)

SSF & Hydrolysis of FW



- Glucoamylases (13.16 U/g) and proteases (53.56 U/g) showed maximum activity at 65 h and 65 % of initial moisture content.
- Invertases showed maximun activity (8.62 U/g) at 65 h and 50% moisture.
- Hydrolysis of 75 g/L of FW was performed using the crude enzyme extraction from SSF and achieved a final production of 153.5 mg/L FAN at 45°C and 19 g/L glucose at 55°C after 50 h.

Conclusions

- ✓ Results showed the high potential of FW as feedstock for biodiesel, hydrolytic enzymes production through SSF and fermentation media production through hydrolysis.
- ✓ Future work will focus on optimization of hydrolysis process of the proposed biorefinery concept and its utilization as fermentative feedstock for microbial oil production.

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Acknowledgements

This research was supported by the Spanish Ministry of Economy and Competitiveness (ENE2013-47769R).