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Integral use of pectin-rich by-products in a biorefinery context: A holistic approach

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ABSTRACT

There is no doubt that in recent years there has been a growing interest in the use of by-products from the agri-food industry with the aim of achieving zero waste. In this context, pectin is a valuable ingredient obtained from vegetable and fruit by-products whose extraction may be a problem rather than a solution. It was not until 2015 when studies began to be published on the need to avoid residues derived from pectin extraction. We show an exhaustive analysis of the existing bibliography on the possible ways for comprehensive use of these residues from a biorefinery approach. In addition, we have carried out a bibliometric analysis of 27 articles reporting by-product composition and high-value ingredient recovery, making a comparison of specific biorefinery applications described in selected articles. A general overview is provided on valorisation strategies, considering the carbohydrate composition (mono-/disaccharides, pectin, cellulose and hemicellulose) of fruit and vegetable by-products. Integrated utilisation strategies should start with the recovery of compounds soluble in organic solvents (essential oils, polyphenols, carotenoids, etc.), followed by the recovery of pectin and subsequent use of depectinised residues. Biofuels (bioethanol, biogas or solid biofuel) can be obtained by transforming the secondary flows by physicochemical or biological processes such as ethanolic fermentation or anaerobic digestion. Other fermentation processes allow organic acids such as succinic, lactic, butyric and mucic acids to be obtained, as well as other compounds such as bacterial cellulose and pullulan. The role of up to 40 microbial glycosidases in fermentation involved in biorefinery applications was also highlighted. Finally, other uses of waste biomass such as composting or animal feed are considered. Although most of the research has been carried out on citrus, this review points out that, despites the differences, studies with these by-products can help in the efficient utilisation of other types of agri-food wastes.

1. Introduction

Since the initial production of pectin from apple pomace in Germany in the early 20th century (May, 1997), the market for pectin evolved significantly due to the increasing demands of the food industry and consumers towards functional food products. Consumers want ingredients in their food products that they can recognise and that are natural in origin (Laughman, 2018), pectin being a nature-derived texturiser with greater functionality as compared to other hydrocolloids (Zhang et al., 2020). It is believed that pectin will have an interesting trade market in the coming years as compared to some other hydrocolloids because of its positive effect at low quantities, relatively low prices and safety (Moslemi, 2021). Moreover, to satisfy the wishes of consumers, the food industry is keen to find low-calorie and low-fat food ingredients. In this sense, pectin can be dispersed in water and can act as a fat replacer in a huge number of processed products (Ciriminna et al., 2016). Pectin is broadly used in the food industry to enhance the desired texture in foods and beverages, among other uses. Pectin has the Generally Recognized as Safe (GRAS) designation in the US and is an accepted food additive in the EU, coded E440. In the last decade, this polysaccharide is considered an essential ingredient in the production of fruit juices, soy drinks and yogurts, and to stabilise acidic proteins during heating (Muñoz-Almagro et al., 2021).

In cosmetics, pectin is widely used as an effective stabiliser in creams, lotions, gels, shampoos and hair tonics, and is used as a skin

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List abbreviations

A. niger	Aspergillus niger
A. pullulans	Aureobasidium pullulans
A. succinogenes	Actinobacillus succinogenes
B. coagulans	Bacillus coagulans
C. tyrobutyricum	Clostridium tyrobutyricum
CA	correspondence analysis
CAZy	Carbohydrate-Active enZYmes Database
COVID-19	coronavirus disease of 2019
CW	citrus waste
DS	dry solids
EAE	enzyme-assisted extraction
EFSA	European Food Safety Authority
GalA	galacturonic acid
GRAS	Generally Recognized as Safe
GWP	global warming potential
HG	homogalacturonan
K. sucrofermentans	Komagataeibacter sucrofermentans
LDW	liquid depectinised wastes
LPMOs	lytic polysaccharide monooxygenase
MAE	microwave-assisted extraction
MTBE	methyl *tert*-butyl ether
MW	microwave
NCBI	National Center for Biotechnology Information
OPW	orange peel waste
P. kudriavzevii	Pichia kudriavzevii
PCA	principal components analysis
POS	pectic oligosaccharides
PPW	pomegranate peel waste
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
RAKE	Rapid Automatic Keyword Extraction
RG I	rhamnogalacturonan I
RG II	rhamnogalacturonan II
S. cerevisiae	*Saccharomyces cerevisiae*
SBP	sugar beet pulp
SDGs	Sustainable Development Goals
SDW	solid depectinised wastes
SWE	subcritical water extraction
T. reesei	Trichoderma reesei
UAE	ultrasound-assisted extraction
US	ultrasound
USD	United States dollar
XG	xylogalacturonan
XOS	xylo-oligosaccharides

anti-aging agent (Lebreton-Decoster et al., 2011). In medicine, pectin also has a number of applications, such as drug delivery, a wound healing formula and in colostomy devices (Ciriminna et al., 2016). In addition, due to its benefits on blood cholesterol reduction, post-prandial glycaemic response and potential for the treatment and prevention of diabetes, pectin has also been recommended by the European Food Safety Authority (EFSA) as an excellent ingredient (Muñoz-Almagro et al., 2021).

As result of its diverse and numerous food applications, pectin is considered an attractive investment, being industrialised by companies such as CP Kelco, Cargill, Danisco/Dupont, FMC Biopolymers, Herbstreith & Fox, Naturex/Obipektin, Yantal Andre Pectin, among others. This polysaccharide contributed meaningfully to the worldwide hydrocolloids market with an estimated value of $964 million retailed in 2015 (Grand View Research, 2017). This market is expected to grow at a rate of 5–6% per year, while pectin consumption worldwide is currently estimated at 45,000 tonnes (Marić et al., 2018; Muñoz-Almagro et al., 2021; Raji et al., 2017). On the other hand, pectin is one of the major constituents of the plant cell wall, and is strongly linked to other biopolymers such as cellulose and hemicellulose. Therefore, pectin extraction methods at an industrial level usually involve prolonged time, high temperature and the use of mineral acids, generating large amounts of acid wastewater and solid residues (Gerschenson et al., 2021). Although there are a plethora of studies on the production of pectin under the so-called green chemistry using organic acids, such as citric, malic and lactic acid, the fact is that this polysaccharide is still obtained at the industrial level under traditional conditions (Adiletta et al., 2020; Marenda et al., 2019).

Societal concern about environmental sustainability has risen significantly over the past decades. Therefore, attention has been paid to the re-valorisation of natural resources in order to obtain functional ingredients. Sustainable biomass processing falls under the "biorefinery" concept (Awasthi et al., 2021; Clauser et al., 2021; Naik et al., 2010). The agri-food sector needs major changes, improving the valorisation of food waste, in order to achieve the targets of the United Nations Sustainable Development Goals (SDGs) 2: Zero Hunger and 12.3: "reduce food losses along production and supply chains, including post-harvest losses" by 2030 (United Nations web portal, 2016). But it should be also noted that the principles of sustainability and sustainable development have undergone changes in response to the current pandemic. In a recent paper, Ranjbari et al. (2021) identify, as one of the main research avenues for making the most of sustainability transition opportunities in the wake of COVID-19, a focus on SDG 12, among others, so that the transition to sustainability in the agri-food sector can be accelerated in the context of the circular bioeconomy.

Biomass can be considered as any organic substance derived directly or indirectly from the process of photosynthesis. Due to the heterogeneity of materials, use and origin, the definition of biomass varies (Tursi, 2019). Agri-food waste consists mainly of lignocellulosic material (cellulose, hemicellulose and lignin) from the plant cell wall. The primary cell wall also consists of a significant amount of pectic polysaccharides that provide structural support in the soft tissues of dicotyledons, while hemicellulose is more abundant in the secondary plant cell walls (Atmodjo et al., 2013). In a biorefinery concept, it is desirable that the feedstock can be transformed into diverse kinds of biofuels, biochemicals and biomaterials (Adiletta et al., 2020; Brachi et al., 2017; Tursi, 2019). Waste generated during food production and processing can be considered feedstock for biorefinery processes, as this waste is generated in a more concentrated and less variable form (Pfaltzgraff et al., 2013). Although seasonality is an issue, numerous examples suggest the feasibility of using plant biomass and agri-food waste as feedstock for biorefining regardless of supply and seasonality, as they constitute cheap non-food materials (Clauser et al., 2021; Naik et al., 2010). Specific applications reported include apple and citrus residues (Awasthi et al., 2021; Zema et al., 2018). However, some authors claim that food waste recovery methods within an integrated "zero waste" concept are at an early stage of development. More studies dealing with a broader range of food matrices and industrial by-products are needed to scale up these methods on an industrial scale (Cristóbal et al., 2018). Thus, the production of pectin through the combination of green chemistry and biorefinery should be a challenge for industries in the agri-food sector (Adiletta et al., 2020; Brachi et al., 2017).

The present review summarises all literature available on the integral valorisation of pectin-rich fruit and vegetable by-products. To our knowledge, this holistic approach has not been covered by other review articles published in the last year (Table 1) nor in previous years. Therefore, valorisation strategies other than the production of pectin as a functional ingredient are summarised. These strategies are based on

Table 1
Main revisions about pectin and biorefinery published since 2021.

Author	Title	Waste/by-product	Main Topic	Integral use	Pectin Topic
Cui et al. (2021)	Pectins from fruits: Relationships between extraction methods, structural characteristics, and functional properties	Fruit waste	Pectin, extraction and characterisation	No	Only pectin
Khubber et al. (2021)	Structural-functional Variability in Pectin and Effect of Innovative Extraction Methods: An Integrated Analysis for Tailored Applications	Fruit and vegetable waste	Pectin, extraction and characterisation	No	Only pectin
Gerschenson et al. (2021)	Pectins obtained by ultrasound from agroindustrial by-products	Fruit and vegetable processing waste	Pectin, coextraction antioxidants	No	Only pectin
Kumar, Tomar, Saurabh, Sasi, Punia et al. (2021)	Delineating the inherent functional descriptors and biofunctionalities of pectic polysaccharides	No waste	Structural and functional characteristics of pectin	No	Only pectin
Cano-Lamadrid and Artés-Hernández (2022)	By-products revalorization with non-thermal treatments to enhance phytochemical compounds of fruit and vegetables derived products: A review	Fruits and vegetables waste	Phytochemicals and pectin extraction	No	Important
Gavahian et al. (2021)	Emerging technologies to obtain pectin from food processing by-products: A strategy for enhancing resource efficiency	Fruit and vegetable waste	Pectin extraction	No	Important
Rifna et al. (2021)	Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: A review	Fruit and vegetable waste	Extraction of bioactive compounds	No	Important
Manhongo et al. (2022)	Current status and opportunities for fruit processing waste biorefineries	Fruit processing waste	Production, composition. Potential products	Important. Biorefinery process diagrams	Important
Awasthi et al. (2021)	A critical review on the development stage of biorefinery systems towards the management of apple processing-derived waste	Appel waste	Direct use, pectin and polyphenolic extraction and biofuel production	Direct use	Important
Casa et al. (2021)	A brief overview on valorisation of industrial tomato by-products using the biorefinery cascade approach	Tomato waste	Extraction and application of high value compounds, biofuels	Theoretical biorefinery model	Important
Karimi et al. (2021)	Bioactive compounds from by-products of eggplant: Functional properties, potential applications and advances in valorisation methods	Eggplant	Production, composition. Potential products	1 example	Important
Xiao et al. (2021)	Utilisation of pomelo peels to manufacture value-added products: A review	Grapefruit peel waste	Extraction and potential uses, focusing on polyphenols and pectin	No	Important
Yadav et al. (2022)	Integrated biorefinery approach to valorize citrus waste: A sustainable solution for resource recovery and environmental management	Citrus waste	Production, composition. Potential products, integrated process	Important. Biorefinery process	Important
Shrestha et al. (2021)	Different Facets of Lignocellulosic Biomass Including Pectin and Its Perspectives	Agricultural and forest-based industries	Composition, pretreatments. Potential products	No	Secondary
Sharma et al. (2021)	Sustainable processing of food waste for production of bio-based products for circular bioeconomy	Food waste	Potencial utilisation, bioactive compounds, biofuels and others	Simplified theoretical diagram for integrated waste recovery	Secondary
Kumar, Srivastav, and Sharanagat (2021)	Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review	Fruit and vegetable processing waste	US parameters and extraction of bioactive compounds	No	Secondary
Panwar et al. (2021)	Recent Trends on the Valorisation Strategies for the Management of Citrus By-products	Citrus waste	Production, composition. Economic and environment-friendly valorisation strategies	Generalized scheme for the valorisation of citrus waste	Secondary
Anticona et al. (2020)	High biological value compounds extraction from citrus waste with non-conventional methods	Citrus waste	Extraction methods applicate at high value compounds	No	Secondary
Mohsin et al. (2021)	Advances in sustainable approaches utilizing orange peel waste to produce highly value-added bioproducts	Orange peel waste	Production, composition. Potential products	Limited examples of pectin and integral utilisation	Secondary
Duan et al. (2021)	Apple orchard waste recycling and valorisation of valuable product-A review	Apple orchard waste	Production. Potential products and uses	No	Secondary
Usmani et al. (2022)	Valorisation of sugar beet pulp to value-added products: A review	Sugar beet pulp	Valorisation of SBP. Methods. Products	Limited examples of pectin and integral utilisation	Secondary
Puligundla and Mok (2021)	Valorisation of sugar beet pulp through biotechnological approaches: recent developments	Sugar beet pulp	Production of value-added products via biotechnological approaches	Limited examples of pectin and integral utilisation	Secondary
Calcio Gaudino et al. (2021)	Sono- And mechanochemical technologies in the catalytic conversion of biomass	Lignocellulosic material, especially cellulose	Process to release fermentable sugars	No	No
Usmani et al. (2022)	Minimizing hazardous impact of food waste in a circular economy – Advances in resource recovery through green strategies	Food waste	Anaerobic digestion	Secondary	No
El Barnossi et al. (2021)			Production, composition. Potential products	No	Only cited

(*continued on next page*)

Table 1 (*continued*)

Author	Title	Waste/by-product	Main Topic	Integral use	Pectin Topic
	Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review	Tangerine, banana and pomegranate peels			
Mahato et al. (2021)	Biotransformation of citrus waste-i: Production of biofuel and valuable compounds by fermentation	Citrus waste	Integral use for biofuel	Yes	No pectin extraction
Jeong et al. (2021)	Recent advances in the biological valorisation of citrus peel waste into fuels and chemicals	Citrus peel waste	Fermentative processes for biofuels and chemicals from pectin-derived sugars	Yes	No pectin extraction

the utilisation of the different depectinised side streams, in an attempt to afford solutions to the burgeoning problems related to its disposal. The production of phenolic compounds, essential oils, bioethanol, biofuel, organic acids and other compounds of interest from these pectin-rich by-products has also been summarised (Table 2). By-product composition (cellulose, hemicellulose, lignin and protein contents) was then correlated to product yields. Finally, a complementary analysis of glycosidase activity involved in the microbial fermentation of plant waste as a biorefinery strategy was carried out.

2. Methods

2.1. Article selection for comparative study

To select the articles included in this systematic review, a naïve search for papers listed in three different databases (PubMed, Scopus and Web of Science) was first performed. This search contained five conceptual terms involving "pectin", "by-product", "valorisation", "extraction" and a more specific conceptual term involving several products and processes that may be associated to by-product biorefinery (biofuel, biogas, ethanol, compost, fermentation). For this purpose, an advanced search option with Boolean operators was used. The format of this initial search was adjusted to each database (Supplementary Material Table S1). A Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) object describing the full article selection process was also generated (Supplementary Material Fig. S1).

Bibliographical information from each article was processed using different mathematical functions implemented in R v3.6.2 to reduce investigator bias in keyword selection and to monitor novel research trends. Results from individual searches were merged using revtools v0.4.1 package (Westgate, 2019). Duplicated articles were filtered by title using synthesisr v0.3.0 library (Westgate & Grames, 2020) leading to 47 original articles. Keywords were then extracted and new keywords were generated from article titles and abstracts. These new keywords reflect those terms that are present in at least two article titles/abstracts and were generated using the *Rapid Automatic Keyword Extraction* (RAKE) method implemented in litsearchr v1.0.0 package (Grames et al., 2019). In addition, a co-occurrence network of article keywords and new keywords extracted from article titles and abstracts was computed using litsearchr v1.0.0 and ggrapgh v2.0.1 packages (Grames et al., 2019; Pedersen, 2020). A graphical representation of the co-occurrence network is provided in Fig. 1. As can be seen, some associations between terms central to the topic suggest the following research lines: 1) valorisation of waste biomass to obtain valuable products using techniques like solvent extraction, 2) optimisation of enzymatic hydrolysis using cellulases by central composite designs to process large amounts of agricultural waste as an integrated biorefinery strategy (Fig. 1). On the other hand, associations between terms that are closely related to the above-mentioned research lines reveal the following applications:

1) Study of energy balances in biorefinery approaches for pectin extraction and recovery of the remaining solid,
2) Anaerobic digestion of organic matter including dietary fibre and citrus waste (CW),
3) Biorefinery approaches to mitigate the environmental impact of the agricultural waste chain,
4) Biorefinery platforms based on the application of commercial enzyme preparations and fermentation using *Actinobacillus succinogenes*
5) Application of commercial enzymes for sugar release,
6) Analysis of compounds of interest using anion exchange chromatography. These associations highlight specific applications reported in some of the articles whereas most of the studies involve enzymatic processes to recover high-value ingredients.

As expected, the most relevant terms included green chemistry, waste processing and valorisation, as well as some bioactive ingredients that may be obtained from these sources such as phenolic compounds and pectic polysaccharides (Supplementary Material Fig. S2). Other relevant terms highlight biotechnological valorisation based on anaerobic digestion and fermentative processes using specific bacterial species. These terms also indicate the predominance of cellulolytic enzymes and commercial preparations to degrade complex polysaccharides (Fig. 1 and Supplementary Material Fig. S2). Once all relevant terms were extracted from articles to elucidate existing research trends, an additional bibliographic search was performed. This second search was structured into four conceptual terms: 1) pectin (the main compound of interest), 2) green chemistry and waste valorisation, 3) extraction, 4) bio-based products obtained (Supplementary Material Table S1). Taking into account the associations described in the co-occurrence network (Fig. 1 and Supplementary Material Fig. S2), additional keywords that fall under one of these four concept categories were added (Supplementary Material Table S1).

The results from final bibliographic research (last accessed October 19, 2021) were processed as previously described leading to 2883 articles filtered by title. These articles were further screened to select those directly related to biorefinery applications as well as carbohydrate and bio-based ingredient obtainment (Supplementary Material Fig. S1). Articles screened were then manually curated to select those reporting fruit and vegetable by-product composition expressed as cellulose, hemicellulose, lignin and protein contents (Table 2 and Supplementary Material Fig. S1). This led to a total of 27 articles describing pectin extraction in a biorefinery context where associations between by-product composition and product yields may be established.

2.2. Bibliometric analysis of articles reporting by-product composition and recovery of high-value ingredients

Selected articles were mainly single country publications, with China and Brazil the most productive countries. Most productive authors published 2–3 articles in 2018 and 2019. To go deeper into the specific biorefinery applications developed in these articles, text analysis was performed using natural language processing tools implemented in quanteda v2.1.2 package (Benoit et al., 2018). A word cloud plot of the most frequent terms (those showing a frequency higher than 6 in at least 3 articles, Supplementary Material Fig. S3A) revealed a large number of

Table 2
Carbohydrate composition (mono-/disaccharides, cellulose, hemicellulose), lignin and protein content of fruit and vegetable by-products from various sources. Pectin has been obtained from these substrates using several extraction methods leading to different pectin characteristics (yield; degree of methyl-esterification, DM; galacturonic acid, GalA and neutral sugar contents). (1) Pectic oligosaccharides yield.

Fraction	Author	Source	Mono- and disaccharides (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Protein (%)
1	Adiletta et al. (2020)	Beet	8.1	21.7	51.8	4.5	7.2
2	Alexandri et al. (2019)	Beet	7.1	23.0	19.5	2.6	9.6
3	Banerjee et al. (2018)	Mango		23.0	3.3	16.0	
4	Alves de Oliveira et al. (2020)	Beet	8.7	21.5	31.6	2.1	9.7
5	Gonzalez-Garcia et al. (2018)	Beet		25.0	35.0	2.0	15.0
6	Gonzalez-Garcia et al. (2018)	Beet		25.0	35.0	2.0	15.0
7	Encalada et al., 2019	Carrot	1.6	10.1	30.0	4.2	5.9
8	Encalada, Pérez, Calderón, et al. (2019)	Carrot	1.6	10.1	30.0	4.2	5.9
9	Encalada, Pérez, Calderón, et al. (2019)	Carrot	1.6	10.1	30.0	4.2	5.9
10	Ma et al. (2019)	Apple	24.5	17.7	10.9	15.4	
11	Ortiz-Sanchez et al. (2021)	Citrus	30.6	30.2	9.4	5.1	4.9
12	Patsalou et al. (2020)	Citrus		22.5	8.1	0.7	
13	Pourbafrani et al. (2010)	Citrus	22.9	22.0	11.1	2.2	6.1
14	Rico, Gullón, and Yáñez (2020)	Melon	36.9	14.2	7.7	11.5	11.1
15	Satari et al. (2017)	Citrus	33.2	8.8	13.6	2.0	
16	Senit et al. (2019)	Citrus	35.0	19.0	14.0		5.8
17	Talekar et al. (2018b)	Pomegranate		16.8	11.7	21.2	4.0
18	Talekar et al. (2018b)	Pomegranate		16.8	11.7	21.2	4.0
19	Talekar et al. (2018a)	Pomegranate		17.6	13.0	22.2	5.0
20	Jahn et al. (2020)	Beet		29.4	34.8	12.8	
21	Jeong et al. (2013)	Rapeseed		37.8	37.8	12.3	33.7
22	Kundu et al. (2021)	Citrus	34.2	28.6	18.6	2.1	
23	Pereira et al. (2021)	Banana	26.8	20.7	17.3	31.6	
24	Tsouko et al. (2020)	Citrus	50.0	9.2	5.4	1.2	6.6
25	Vaez et al. (2021)	Citrus		36.5	17.2	7.7	
26	Wang et al. (2021)	Pomegranate		17.6	13.0	22.2	5.0
27	Yang et al. (2020)	Citrus	57.1	22.6	6.0	8.6	
28	Yang et al. (2020)	Citrus	53.2	37.1	11.0	7.5	
29	Hamley-Bennett et al. (2016)	Beet		26.0	26.0	2.0	15.0
30	Kazemi et al. (2019)	Eggplant		16.0	4.3	1.8	3.4
31	Kyriakou et al. (2020)	Citrus	53.2	37.1	11.0	7.5	
32	Ortiz-Sanchez et al. (2020)	Citrus	30.6	30.2	9.4	5.1	4.9

Fraction	Author	Source	Pectin/POS extraction method	Pectin yield (%)	DM (%)	GalA (%)	Pectin neutral sugars (%)
1	Adiletta et al. (2020)	Beet	Organic acid	25.0	69.0		
2	Alexandri et al. (2019)	Beet	Inorganic acid	30.3			
3	Banerjee et al. (2018)	Mango	Hydrothermal	27.2	89.0		
4	Alves de Oliveira et al., 2020	Beet	Organic acid	29.2			
5	Gonzalez-Garcia et al. (2018)	Beet	Hydrothermal	25.4(1)			
6	Gonzalez-Garcia et al. (2018)	Beet	Enzymatic	20.6(1)			
7	Encalada et al., 2019	Carrot	Ultrasound + enzymatic	27.1	24.0	45.0	52.0
8	Encalada, Pérez, Calderón, et al. (2019)	Carrot	Ultrasound	35.4	24.0	40.0	45.0
9	Encalada, Pérez, Calderón, et al. (2019)	Carrot	Alkaline	23.0	24.0	39.0	36.0
10	Ma et al. (2019)	Apple	Organic acid	19.6			
11	Ortiz-Sanchez et al. (2021)	Citrus	Organic acid	10.4			
12	Patsalou et al. (2020)	Citrus	Inorganic acid	19.9			
13	Pourbafrani et al. (2010)	Citrus	Inorganic acid	19.4	77.6		
14	Rico, Gullón, and Yáñez (2020)	Melon	Hydrothermal	9.4(1)			
15	Satari et al. (2017)	Citrus	Inorganic acid	23.2	68.1		
16	Senit et al. (2019)	Citrus	Inorganic acid	19.6	70.0	60.0	31.0
17	Talekar et al. (2018b)	Pomegranate	Ultrasound	24.8	68.5	72.0	
18	Talekar et al. (2018b)	Pomegranate	Inorganic acid	24.4	60.2	65.0	
19	Talekar et al. (2018a)	Pomegranate	Hydrothermal	20.9	74.0	72.0	
20	Jahn et al. (2020)	Beet	Inorganic acid	24.6			
21	Jeong et al. (2013)	Rapeseed	Enzymatic	6.2	44.9	64.2	
22	Kundu et al. (2021)	Citrus	Enzymatic	12.7	74.1	70.8	
23	Pereira et al. (2021)	Banana	Organic acid	8.0			
24	Tsouko et al. (2020)	Citrus	Organic acid	16.3	72.0		
25	Vaez et al. (2021)	Citrus	Inorganic acid	24.7	69.0	70.2	
26	Wang et al. (2021)	Pomegranate	Ultrasound + enzymatic	24.8			
27	Yang et al. (2020)	Citrus	Fermentation	26.1(1)		62.5	37.5
28	Yang et al. (2020)	Citrus	Fermentation	15.7(1)		50.5	49.5
29	Hamley-Bennett et al. (2016)	Beet	Hydrothermal	32.9(1)			
30	Kazemi et al. (2019)	Eggplant	Organic acid	26.1	60.2	69.7	
31	Kyriakou et al. (2020)	Citrus	Inorganic acid	30.5			
32	Ortiz-Sanchez et al. (2020)	Citrus	Organic acid	15.9	66.8	6.6	

Fraction	Author	Phenolic compound yield (%)	Essential oil yield (%)	Microorganism used for fermentation	Type of biotechnological process	Fermentation products
1	Adiletta et al. (2020)					
2	Alexandri et al. (2019)	7.9		*Actinobacillus succinogenes*	Enzyme preparation + Fermentation	Succinic acid

(*continued on next page*)

Table 2 (*continued*)

Fraction	Author	Phenolic compound yield (%)	Essential oil yield (%)	Microorganism used for fermentation	Type of biotechnological process	Fermentation products
3	Banerjee et al. (2018)	18.0				
4	Alves de Oliveira et al. (2020)			*Bacillus coagulans*	Fermentation	Lactic acid, feed
5	Gonzalez-Garcia et al. (2018)					
6	Gonzalez-Garcia et al. (2018)					
7	Encalada et al., 2019					
8	Encalada, Pérez, Calderón, et al. (2019)					
9	Encalada, Pérez, Calderón, et al. (2019)					
10	Ma et al. (2019)					
11	Ortiz-Sanchez et al. (2021)		0.8	Anaerobic digestion	Fermentation	Biofuel/biogas
12	Patsalou et al. (2020)		2.2	*Actinobacillus succinogenes*	Enzyme preparation + Fermentation	Succinic acid
13	Pourbafrani et al. (2010)		4.5	*Saccharomyces cerevisiae*	Fermentation	Ethanol
14	Rico, Gullón, and Yáñez (2020)	0.4				
15	Satari et al. (2017)			*Mucor indicus*	Enzyme preparation + Fermentation	Ethanol, fungal biomass
16	Senit et al. (2019)	1.6	2.5			
17	Talekar et al. (2018b)	11.9				
18	Talekar et al. (2018b)	11.9				
19	Talekar et al. (2018a)	11.8		*Saccharomyces cerevisiae*	Enzyme preparation + Fermentation	Ethanol
20	Jahn et al. (2020)					
21	Jeong et al. (2013)		9.9			
22	Kundu et al. (2021)		4.2	*Pichia kudriavzevii/ Saccharomyces cerevisiae*	Enzyme preparation + Fermentation	Ethanol
23	Pereira et al. (2021)	0.8				
24	Tsouko et al. (2020)	0.6	0.7	*Komagataeibacter sucrofermentants*	Enzyme preparation + Fermentation	Bacterial cellulose
25	Vaez et al. (2021)			*Saccharomyces cerevisiae*	Fermentation	Ethanol
26	Wang et al. (2021)	12.2		*Clostridium tyrobutyricum*	Enzyme preparation + Fermentation	Butyric acid
27	Yang et al. (2020)			*Aspergillus niger* gene in *Pichia pastoris*	Enzyme preparation + Fermentation	POS
28	Yang et al. (2020)			*Aspergillus niger* gene in *Pichia pastoris*	Enzyme preparation + Fermentation	POS
29	Hamley-Bennett et al. (2016)			*Saccharomyces cerevisiae*	Enzyme preparation + Fermentation	Ethanol
30	Kazemi et al. (2019)	20.2		*Aureobasidium pullulans*	Enzyme preparation + Fermentation	Pullulan
31	Kyriakou et al. (2020)			*Saccharomyces cerevisiae*	Enzyme preparation + Fermentation	Ethanol
32	Ortiz-Sanchez et al. (2020)			*Trichoderma reesei*	Fermentation + Fermentation	Biofuel/biogas, mucic acid

articles reporting the acid extraction of pectin. In addition, citrus peel was the most common substrate used in these studies in agreement with the co-occurrence network presented in Fig. 1 and Supplementary Material Fig. S2. Other terms highlight the optimisation of process conditions to achieve high purity and yields of high-value compounds like pectin, phenolic compounds and essential oils, as well as ethanol production. Interestingly, the number of articles reporting the recovery of these bioactive ingredients increased in recent years according to text keyness (relative frequency) analysis that compares the relative frequency of terms from two groups of papers (i.e. time periods before and after 2015) (Supplementary Material Figure Fig. S3B). Similarly, the number of articles reporting by-product characterisation (cellulose, hemicellulose and lignin content), increased after 2015, highlighting the general interest in correlating by-product composition to extraction yields. Some of these recent valorisation approaches involved fermentation processes as indicated in previous modelling steps (Fig. 1 and Supplementary Material Fig. S2). In contrast, studies published before 2015 focused on the optimisation of simple pectin extraction methods (not integrated biorefinery approaches) as well as the production of non-bioactive ingredients (bioethanol, biogas and sugar recovery) (Supplementary Material Fig. S3B).

Topic modelling techniques implemented in bibliometrix v3.0.4 package (Derviş, 2019) revealed three major topics in 27 articles selected (Fig. 2A): 1) studies reporting by-product cellulose contents and cellulose production, 2) studies describing the applications of enzymatic hydrolysis and microbial fermentation, 3) studies reporting ethanol production in the context of pectin extraction processes. The conceptual structure map generated by correspondence analysis (CA) indicated that these three topics are well discriminated and correspond to different research lines (Fig. 2B).

3. Obtaining pectin

The most accepted model of pectin structure includes the structural domains of homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II) and, in some cases, xylogalacturonan (XG) (Dranca & Oroian, 2018). HG represents 65% of pectin molecules, with a linear backbone composed of α-(1,4)-D-galacturonic acid (GalA), which may be partially methyl-esterified in C6, or acetylated in O-3 and/or O-2. RG I is 20–35% of molecules; the main backbone has units of GalA and rhamnose. This chain of repeating disaccharides $[\rightarrow 4)\alpha\text{-D-GalA-}(1\text{–}2)\alpha\text{-L-rhamnose-}(1\rightarrow]_n$ may have side chains of molecules of L-arabinose and D-galactose. Finally, RG II represents 10% of pectin molecules and is a well-preserved and exceptionally complex domain, where the main part is HG with four heteropolymers including rare monosaccharides such as D-apiose, L-aceric acid, L-fucose, 3-deoxy--D-lyxo-heptulosic acid and 2-keto-3-deoxy-D-manno-octulosonic acid (Atmodjo et al., 2013). The monosaccharide composition, branched

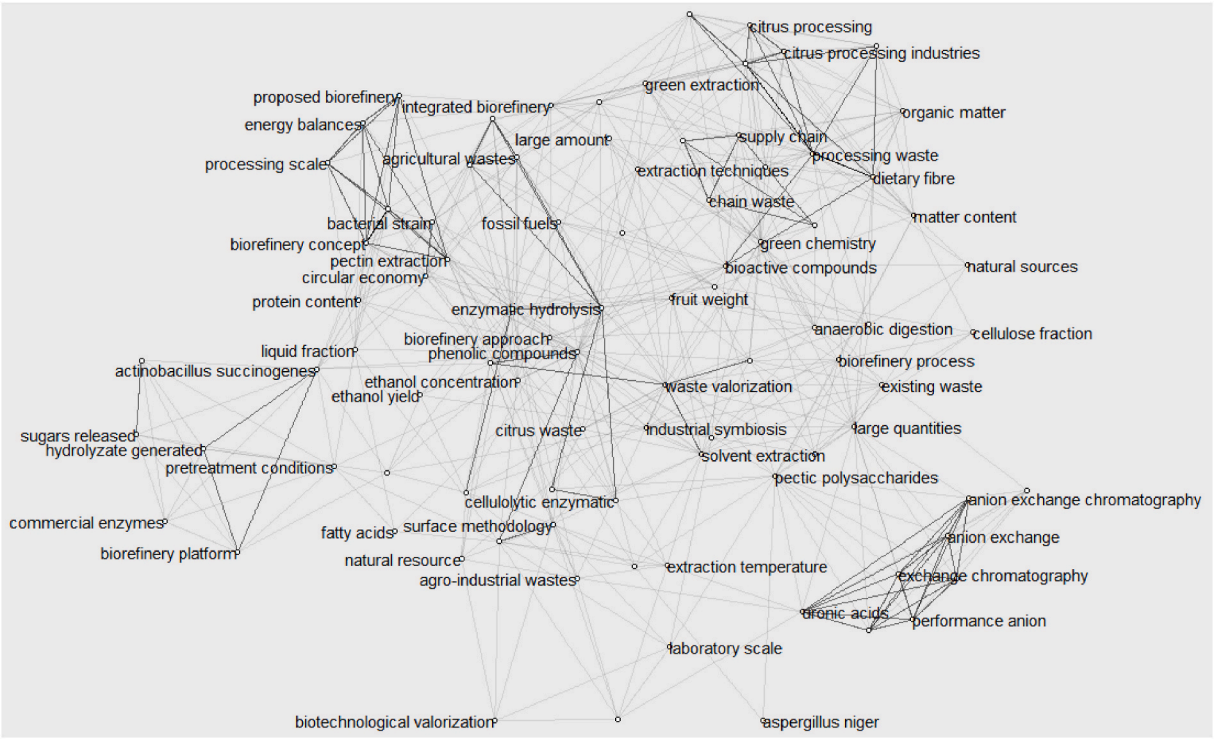

Fig. 1. Co-occurrence network of article keywords and new keywords extracted from article titles and abstracts. Those terms that appear near the center of the graph and that are linked to each other by darker lines are the most relevant for the overall topic while terms that appear at the periphery of the graph and linked to it only by faint lines are related to, but not part of, the main research topic (pectin obtainment from vegetable by-products in a biorefinery context).

type, esterification and molecular weight of pectin differ with the raw material and extraction techniques and conditions, which are key factors determining the physicochemical characteristics of pectin (Dranca & Oroian, 2018).

Apple pomace, citrus peel and sugar beet pomace are traditionally used as feedstock to obtain pectin industrially, which results in optimal yields of 19%, 37% and 24%, respectively (Marenda et al., 2019; Pacheco et al., 2019). However, new sources have been proposed with excellent results such as sunflower (Muñoz-Almagro et al., 2018, 2020) and artichoke by-products (Sabater et al., 2018, 2020). As aforementioned, pectin is widely present in the middle lamella and primary cell walls glued together with other polysaccharides (Dranca et al., 2020). Therefore, for industrial pectin extraction it is necessary to use extracting agents as water acidified with sulphuric, nitric or hydrochloric acids to break down the cell wall fibre and release pectin chains. Traditional heating extraction requires an acidic pH (2–3), high solid-to-liquid ratio (1:30–1:50), high temperatures (80–90 °C) during a long period (1–5 h) to help pectin dissolution in water (Chan et al., 2017; Grassino et al., 2018; Kang et al., 2015).

The choice of these conventional methods is based on the maximum yields and the gelling properties of pectin obtained, and can result in the enrichment in the amount of GalA and the extensive hydrolysis of neutral sugar side chains present in RG regions (Minjares-Fuentes et al., 2014). However, the toxicity of these strong mineral acids and the environmentally corrosive effluents that they produce are their main problems. Special treatments removing toxic compounds from pectin extracts are required which increases the cost of production (Marić et al., 2018). These limitations have led to other solvents and techniques being considered. In this sense, organic acids such as citric and acetic acid and chelators including citrates, oxalates and polyphosphates can provoke lower depolymerisation of pectin, and are also more adequate from an eco-friendly point of view (Kang et al., 2015). However, these extracting agents are less efficient than mineral acids, due to their lower dissociation constant (Bagherian et al., 2011; Marić et al., 2018).

One of the main complications derived from the extraction of pectin is to clarify if the valorisation of by-products really compensates the energy consumption and the economic demands. In this sense, the shortening of the extraction process could be an adequate option and numerous faster and more sustainable alternative procedures than traditional ones have appeared to obtain pectin (Adetunji et al., 2017; Marenda et al., 2019). The most favourable of these innovative methods comprise microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), subcritical water extraction (SWE) and enzyme-assisted extraction (EAE) (Gharibzahedi et al., 2019; Li et al., 2019; Marić et al., 2018; Rodsamran & Sothornvit, 2019).

In the case of MAE, the pectin extraction depends on the dielectric properties and volume of the solvent, temperature, moisture content and power. The extraction is produced by the direct heating of the microwave-absorbing matrix and/or the heating of a polar solvent to boiling point. The lower processing time, solvent consumption, uniform temperature distribution within the medium, solvent penetration, high pectin yield of high purity in terms of GalA content and reduced size of the equipment are the main positive factors (Adetunji et al., 2017; Bagherian et al., 2011). However, the utilisation of acidified water causes corrosion problems and the subsequent wear of equipment and the microwave (MW) power and irradiation time can give rise to a negative impact on the degree of methyl esterification (Naqash et al., 2017).

The UAE efficiency depends on temperature, frequency, particle size, sonication time and solvent-mass ratio (Wang & Weller, 2006). The mechanism of UAE is based on the collapse of cavitation bubbles near cell walls induced by ultrasound (US) provoking cell disruption, contributing to enhanced solvent entrance into the cells and intensification of mass transfer (Tiwari, 2015). In addition, the shock wave aids in swelling, hydration and the creation of large holes in the cell wall (Marić et al., 2018). UAE can be applied in a bath or in probe units, the latter being more adequate due to the fact that energy is applied on a precise sample area (Marenda et al., 2019). Lower energy consumption,

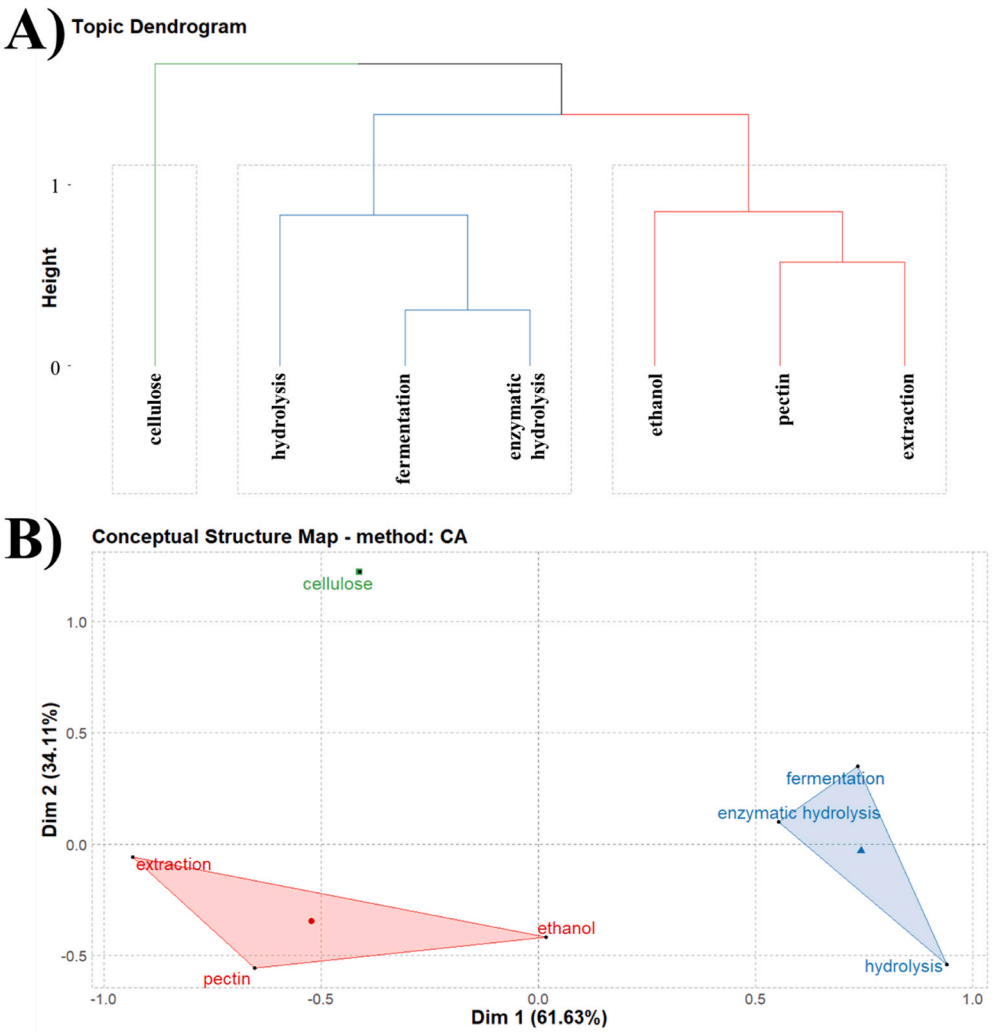

Fig. 2. Keywords comprising the three main research topics from selected articles reporting both by-product characterisation (expressed as cellulose, hemicellulose, lignin and protein contents) and pectin extraction in a biorefinery context (**A**). Conceptual structure maps of topics generated by correspondence analysis (CA). As it can be seen, topics are well discriminated and correspond to different research lines (**B**).

shortened treatment time, less solvent usage, more effective mixing, faster energy and mass transfer, reduced equipment size, a faster response to process extraction control, a faster start-up, increased production, increased safety of the operators, increased yield and better commitment in environmental terms are the principal benefits of the UAE as compared to traditional heating methods (Chemat et al., 2017; Marić et al., 2018; Roselló-Soto et al., 2016). Although UAE does not significantly decrease the solvent amount and, under certain situations, uniformity may not be reached for all the materials dispersed in the solvent, UAE could be the method of choice for the industrial extraction of pectin (Adetunji et al., 2017).

The SWE process involves the application of water at temperatures higher than its boiling point under high pressure to maintain its liquid state. Thus, high diffusion, low viscosity and low surface tension are attained with these elevated temperatures and pressure conditions (Zakaria & Kamal, 2016). Some of the positive effects of this eco-friendly process are the high-quality extracts, shortening of the process and lack of acidic cosolvents (Muñoz-Almagro et al., 2019; Ueno et al., 2008). On the contrary, inappropriate regulation of conditions could give rise to the hydrolysis of the pectin, moreover, the high cost of implementation could also be inconvenient for industrial pectin production.

In EAE, enzymes are used to increase the extraction process by hydrolysing the matrix of the plant cell wall, increasing cell permeability. The high selectivity can lead to high yields. The most commonly used enzymes are cellulases, hemicellulases, xylanases, polygalacturonases, pectin lyases and pectin methyl esterases (Adetunji et al., 2017; Marić et al., 2018; Sabater et al., 2018; Wikiera et al., 2015). It is necessary to take into account the reaction time, type and concentration of enzyme and substrate, temperature, pH value and particle size of feedstock. The low temperatures applied during EAE decrease energy consumption and equipment corrosion (Poojary et al., 2017; Roselló-Soto et al., 2016; Saha et al., 2017). In opposition to conventional methods, EAE does not need an acidic pH and neutralisation resulting in a superior quality of extracted pectin due to the high efficiency and specificity of enzymes. The cost of enzymes is one of the aspects that needs to be improved (Adetunji et al., 2017; Marić et al., 2018; Saha et al., 2017).

Once the extract is obtained, a subsequent purification phase is needed. Thus, the extract is then centrifuged, filtered, precipitated with alcohol, and washed with alcohol water. Another option is purification by membrane separation, an eco-friendly alternative to conventional procedures (Lin et al., 2021; Muñoz-Almagro et al., 2020).

4. Strategies for the valorisation of waste from pectin extraction

Once research trends in pectin extraction and by-product valorisation in a biorefinery context have been presented, the comparison of specific biorefinery applications described in selected articles is shown

(Table 2). These applications can be grouped in four main categories: 1) biofuel production including bioethanol, biogas and solid biofuel; 2) physicochemical transformation of by-products; 3) biotechnological assisted valorisation involving fermentation processes; 4) other uses of residual biomass. A general overview of these four valorisation strategies is provided in this section (Fig. 3). As previously explained, a wide range of bioactive compounds (phytochemicals and secondary metabolites, polyphenols, essential oils, carotenoids) including pectins have been extracted from agri-food waste. These high-value ingredients could be used in the food, cosmetic and pharmaceutical sectors. However, remaining compounds (mainly polysaccharides) could be useful for biofuel and cellulose production as well as for microbial fermentation. Residues from pectin extraction usually comprise two fractions: 1) solid depectinised wastes (SDW) remaining after the solubilisation of pectin from the primary plant cell wall, 2) liquid depectinised waste (LDW) remaining after the isolation of pectin by alcohol precipitation or membrane filtration, consisting mainly of low molecular weight carbohydrates. In biorefinery processes, SDWs are often valorised, whereas LDWs are not always taken into account.

On an industrial scale, the valorisation of pectin extraction wastes can be carried out by physicochemical processes, which are energy-intensive, have low specificity and are not environmentally friendly (Sharma et al., 2021). These disadvantages may be overcome by high-cost enzymatic methods, although the cost is balanced by their excellent efficiency. The market for industrial enzymes increased to 5.9 billion United States dollar (USD) in 2020 and it is expected to reach USD 8.7 billion by 2026, with an annual growth of 6.5% (Sharma et al., 2021). The complete processing of biomass requires the combination of physicochemical treatments with biotransformation, either by using enzymes, fermentation, an anaerobic digestion process or a combination of them. Siles-Lopez et al. (2010) proposed a theoretical scheme for the biorefinery valorisation of orange peel waste (OPW), maximising the value derived from by-products while minimising the amount of residual waste. In CW, in general, the first step is the obtainment of essential oils, followed by pectin extraction. The remaining biomass consists mainly of lignocellulosic material, which allows the growth of microorganisms to generate high value-added products or fuels such as bioethanol or biogas. Another possibility suggested by these authors was the production of industrial enzymes or single-cell proteins, before the final step of using the remaining lignin as an energy source. In 2010, Pourbafrani and co-workers. investigated the production of limonene, pectin, bioethanol and biogas from CW using an integrated process (Pourbafrani et al., 2010). Theoretical biorefinery approaches have been proposed for the integral valorisation of other substrates like pineapple, melon, watermelon and pumpkin (Rico, Gullón, Alonso, & Yáñez, 2020).

4.1. Biofuel production

Biofuels are classified according to whether they are liquid, such as bioethanol, or gaseous, such as biomethane, and are generally produced by fermentation and anaerobic digestion, respectively. The production of biofuels from pectin-rich agri-food by-products has emerged as one of the most strategically important valorisation approaches due to the abundance of fermentable sugars and the low lignin content of pectinous wastes (Xiao & Anderson, 2013). Another advantage of some of these materials, such as pomace from fruit juices, is that they are already collected and partially pre-treated to facilitate the enzymatic deconstruction of plant cell walls (Edwards & Doran-Peterson, 2012). However, pectin is also a recalcitrant cell wall material residue resistant to fermentation by most yeasts, because of its chemical properties. RG-II borate diester cross-linking and HG:HG salt bridges formed by pectins results in increased cell wall recalcitrance. Consequently, down-regulation of pectin increases the accessibility of cell wall biomass to enzymatic degradation (Biswal et al., 2018). In addition, pectin mostly made up of GalA is resistant to fermentation by most yeasts. Even *Saccharomyces cerevisiae* is not able to metabolise the pentoses also present in pectin (Martins et al., 2020). Another possibility to increase the fermentability of cell wall material is its genetic modification, to improve biomass yield and enzymatic saccharification using transgenic plants (Wang et al., 2016). However, it may be easier to pre-extract the compounds of interest. Mohsin et al. (2021) highlighted that biomass valorisation for co-production of bioenergy and high-value, low-volume products (phytochemicals and pectin) has potential economic benefits. These authors also recommended integrated multi-feed fruit and vegetable waste-based biorefineries. Another advantage of this pre-extraction is that the treatments applied usually favour the saccharification process, which is simpler than those required for lignocellulosic material, and mainly focused on hydrothermal and/or enzymatic processes (Zema et al., 2018). On the other hand, the agri-food waste used is subjected to different pre-treatments depending on the type of functional compounds present in the by-product (essential oils, phenolic compounds, pectin) (Panwar et al., 2021). Finally, in

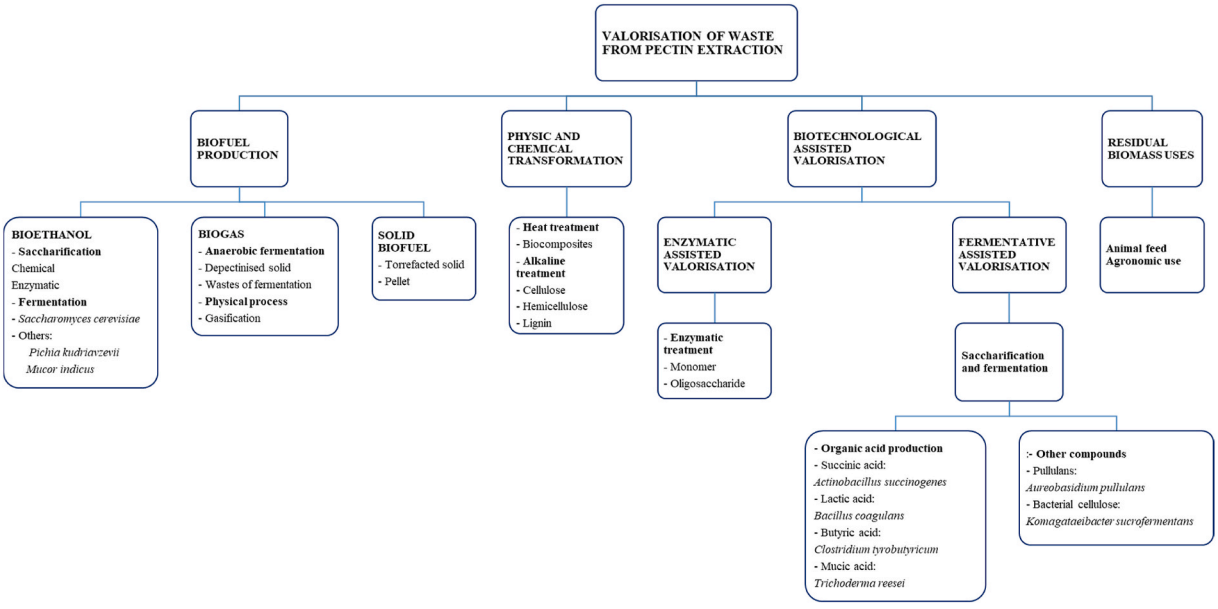

Fig. 3. General scheme of the valorisation of wastes obtained after pectin extraction.

terms of biofuel production, in most cases, microbial fermentation or anaerobic digestion plays a key role and some phytochemical compounds, such as polyphenols and essential oils, have an antimicrobial effect and inhibit the microbiological process. Thus, as mentioned above, citrus peel is rich in organic acids and essential oils. Both compounds are antimicrobial agents, so it is critical to determine the threshold concentration of compounds such as D-limonene that can inhibit microbial growth (Stewart et al., 2013). Widmer et al. (2010) pre-treated CW at 160 °C for 4 min decreasing the limonene content below 0.1%. Therefore, their extraction will favour the subsequent fermentation step (Siles-López et al., 2010).

As mentioned previously, biorefinery processes have been most extensively studied in CWs and, taking into account the differences, studies carried out with these by-products can help in the efficient utilisation of other agri-food wastes. Thus, the current biorefinery strategy for CWs aims to produce bioethanol and biomethane through a multi-step process: 1) extraction of essential oils and pectin; 2) enzymatic hydrolysis of easily hydrolysable waste material; 3) fermentation and/or anaerobic digestion for bioethanol and biomethane production, respectively (John et al., 2017). These authors and Xiao and Anderson (2013) pointed out that the removal and recovery of both D-limonene and pectin from citrus peel are essential for better fermentation and monetary benefits.

4.1.1. Bioethanol

In 2020, the global bioethanol market reached 38 billion and is predicted to reach $76 billion in 2026 (Grand View web portal, 2021). Bioethanol is one of the most efficient biofuels and can be used to improve the octane rating as well as to replace lead as an anti-knock agent for fossil fuels (Zema et al., 2018). Bioethanol is more efficient than gasoline in terms of flame speed, flammability, heat of vaporization and octane number. For this reason, bioethanol is used as a gasoline additive to replace methyl *tert*-butyl ether (MTBE) to increase the octane number. This oxygenated additive increases NO_X emissions and, although highly carcinogenic, is still added to gasoline in many parts of the world. In a recent paper, Panahi et al. (2020) indicate that 7.7–10% ethanol is commonly used to replace MTBE. Currently, an ethanol-gasoline blend programme is exercised in a number of countries and the highest blend (24%) is used in Brazil. Therefore, bioethanol can help many countries tackle air pollution in their large cities and the adverse effects of MTBE on their populations and ecosystems (Panahi et al., 2020).

The most important yeast for bioethanol production is *S. cerevisiae*, however this yeast species cannot naturally catabolise GalA and arabinose, the main sugars present in pectin-rich agri-food waste. Therefore, pectin extraction can be performed as a pre-treatment. Most bioethanol on the market is produced by *S. cerevisiae* from glucose and fructose. Moreover, the presence of pectin in the fermented medium increases the viscosity, therefore distillation would be problematic (John et al., 2017; Zema et al., 2018), although there is equipment available that can solve this problem by decreasing the viscosity of the medium (Widmer et al., 2010).

The production of bioethanol from SDW requires that the present polysaccharides, cellulose and hemicellulose, are hydrolysed by treatment with acids or with enzymes that allow the release of the monosaccharides (saccharification). These monosaccharides will later be converted into bioethanol by the yeasts. The sugar composition of the hydrolysates obtained depends on the starting material and the hydrolysis conditions used (John et al., 2017). Post-enzymatic hydrolysis of pectin-rich by-products gives a mixture containing high levels of GalA and arabinose, which are not fermentable to ethanol by the action of yeasts. In such a case, *E. coli* KO11 (Mahato et al., 2021), an ethanologenic recombinant strain of the bacterium, or *Erwinia chrysanthemi* (Edwards and Doran-Peterson, 2012) are employed to carry out the fermentation. However, GalA fermentation results in the production of equimolar amounts of acetate and ethanol along with CO_2.

When pectin is extracted by dilute acid under a high temperature, the conditions used determine whether or not the SDW need a subsequent enzymatic saccharification step prior to fermentation. In one of the first studies, Pourbafrani et al. (2010) carried out a hydrolysis process with H_2SO_4 0.5%, 15% solids, 150 °C for 6 min, releasing 0.41 g/g dry solids (DS) of sugars. These sugars were converted into bioethanol using baker's yeast (30 °C for 24 h, pH 5 and anaerobic conditions), achieving a bioethanol yield of 0.43 g/g DS. This biorefinery process allowed the production of 39.6 L of bioethanol from 1 tonnes of CW with 20% DS. However, for Kyriakou et al. (2020), using the same acid, at 116 °C, 10 min and 5% solids, extracting pectin from CW required a subsequent enzymatic treatment of the solid residue with cellulase and β-glucosidase/pectinase due to the less severe conditions. These authors also used LDW and immobilised *S. cerevisiae* to produce bioethanol, achieving a bioethanol production of 30.8 g/L from a solution with 100 g/L of initial sugars. This process increased bioethanol production, reduced operating costs and enabled the recyclability of the biocatalyst. In the same line, Vaez et al. (2021) applied H_2SO_4 1% and obtained a better pectin yield at 94 °C during 60 min (24%) and poorer bioethanol production (2.7% v/w) when compared with the treatment at 140 °C and 30 min, obtaining only 3% of pectin and 4.5% v/w of ethanol. Grohman et al. (2013) treated CW with steam to extract pectin fragments, with molecular weight up to 700 kDa, as a value added co-product prior to fermentation for biofuel production.

When water is used as the sole solvent in hydrothermal treatments, the enzymatic saccharification step is always necessary, and cellulases or combinations of cellulases and other enzymes such as β-glucosidase are used. This is the case of the study carried out by Hamley-Bennett et al. (2016) who treated sugar beet pulp (SBP) at 5 bar (152 °C) for 24 min to release pectic oligosaccharides (POS) (83% arabinose, 40% GalA). The cellulose-enriched residual fraction was then subjected to enzymatic digestion using cellulase with secondary activity of cellobiase, β-glucosidase and β-glucanase, releasing all available glucose (18% DS) and lower amounts of arabinose and xylose, producing bioethanol (0.48 g/g of glucose consumed). Talekar et al. (2018a, b) also proposed the utilisation of pomegranate peel waste (PPW) after hydrothermal processing (115 °C, 40 min, 10% solid) and treatment of the SDW with cellulase (30 U/g, pH 4.8, 50 °C, 36 h). In this case, the yield was glucose 177 g/kg DS of PPW, and minor amount of xylose, mannose and arabinose. By fermentation (12% solid, yeast loading 1 g/L, 72 h) they then obtained 80 g bioethanol, reporting an 88% theoretical yield. Under the same conditions used by Talekar et al. (2018a) for polyphenol and pectin extraction, Mazaheri et al. (2021) studied the production of bioethanol from depectinised PPW by a simultaneous saccharification and fermentation process. The optimum process conditions, determined by experimental design, were pH 5.65, temperature 40.3 °C, solids 12.8% w/v, and enzyme dosage 32.3 U/g, under which the maximum amount of ethanol produced was 12.9 g/L, corresponding to 95% of the theoretical yield of ethanol production. Cameron et al. (2016), and Cameron et al. (2017) developed a continuous steam explosion process to utilise CW and fallen citrus affected by huanglongbing infection caused by *Candidatus liberibacter* spp. By steam injection (50–55 psi, 150 °C, 1–3 min) into the CW stream, pectin, phenolic compounds and some other secondary metabolites were recovered. The soluble sugars, present in steam-exploded CW water (23–41% of DS), mainly composed of glucose, fructose and sucrose, and the residual solid (about 15–20% of initial CW), could then be used to produce bioethanol. This research group, after studying the cost-effectiveness of the process, determined that a treatment at 170 °C for 8 min was recommended to maximise the amount of sugars (25–40% DS), essential oil (up to 90% initial content) and flavonoids (4–6% DS) (Dorado et al., 2019). Another consideration is that although *S. cerevisiae* is the most commonly used yeast for bioethanol production, it can also be used in combination with other yeasts. To maximise ethanol production, Kundu et al. (2021) used *S. cerevisiae*, as a hexose-using strain, and *Pichia kudriavzevii*, as a pentose-using strain, to ferment depectinised lemon waste subjected to simultaneous

partial saccharification with a cellulase (pH 4.8; 50 °C, 1 h, 30% w/v). The maximum ethanol yield (12.2% v/v) was obtained when the solids loading, temperature, time and pentose to hexose-using strain ratio were maintained as 30% (w/v), 35 °C, 24 h and 0.5, respectively. The overall efficiency of the process was 69.2%. Valladares-Diestra, Porto de Souza Vandenberghe, et al. (2022) after citric acid-assisted hydrothermal pretreatment (120 °C, 10 min, solids 2% w/v) of cocoa pod husks recovered pectin (19.3%) and XOS (5.2%, from LDW) and fermentable sugars. In a later work the hydrolysed SDW, with a ratio glucose/xylose 4/1, was fermented with *S. cerevisiae* and LDW, with a ratio glucose/xylose 1/1, by *Candida tropicalis*, yielding 7.6% (w/w) of the original waste (Valladares-Diestra, de Souza Vandenberghe, & Soccol, 2022). Regarding the use of other microorganisms, Satari et al. (2017) studied the production of bioethanol with the filamentous fungus *Mucor indicus* from acid-pretreated, limonene- and pectin-free CW. First, the material was subjected to cellulolytic enzymatic hydrolysis for 72 h, yielding 45% (w/w) of glucose of the residual CW solid, with a lower amount of galactose and arabinose. Subsequent ethanolic fermentation by *M. indicus* yields <20% ethanol, probably due to the fact that *M. indicus* used ethanol as a carbon source. Therefore, it was found that this filamentous fungus was suitable for biomass production, but not for ethanol production.

Recently, Manhongo et al. (2021a) conducted a theoretical study on the economic and technical feasibility and environmental impacts of mango waste utilisation in three production scenarios. The first, more basic, was the production of bioethanol, electricity and heat. The second scenario was coupled with pectin extraction by means of subcritical water treatment. The third was with a prior sequential recovery of polyphenols and pectin. According to the economic results and the energy analysis of the three scenarios, the co-production of bioethanol and bioenergy is not profitable. The other scenarios were cost-effective and the co-production of bioenergy and pectin had lower environmental impacts. The incorporation of polyphenol recovery increased the profitability but also the environmental impacts. Consequently, the results suggest that in process implementation decisions, trade-offs must be made between profitability and environmental impacts.

4.1.2. Biogas

In response to the need to minimise the impacts of waste disposal, fruit and vegetables processing waste is currently used to produce animal feed, compost and biogas, another biofuel of great interest, with a global market predicted to reach $50 billion in 2026. It is a mixture of gases, mainly methane (50–70%) which is the energy-rich portion and carbon dioxide (30–50%) (Manhongo et al., 2022). Biogas can be produced from a variety of sustainable substrates by methanogenic bacteria, present in thermophilic anaerobic digestion processes of agri-food waste (Siles-López et al., 2010). Anaerobic digestion of whole or partial agri-food waste is a promising and sustainable option for biogas generation due to the high methanogenic potential of this waste (Ortiz--Sanchez et al., 2020; Panwar et al., 2021). Despite the huge potential, biogas technologies are not economically attractive due to their high cost and an erratic supply of feedstock. Integrated biorefineries for co-producing biogas with high-value compounds including pectin, polyphenols, succinic acid, and essential oils can be more attractive (Manhongo et al., 2022). In Pourbafrani et al. (2010), as mentioned above, the last step of the integrated process for the utilisation of OPW involved biogas production from the residual liquid and solid material after bioethanol production by anaerobic digestion (3% solids, 55 °C, 50 days). This biorefinery process, from 1 ton of dry matter of CW, allowed the production of 44.5 L of limonene, 194 kg of pectin, 198 L of bioethanol (0.43 g/g of sugar consumed), and 180 m^3 of biomethane. Vaez et al. (2021) followed a similar process, but without limonene separation, obtaining 271 kg of pectin, 29.4 L of bioethanol and 40 m^3 of biomethane. This was possible by combining strong pre-treatment conditions (1% H_2SO_4, 140 °C, 30 min) and anaerobic digestion. Dissimilarities in biorefinery applications may be due to the different initial treatment to obtain pectin, milder in the later process, which allows a higher pectin yield to be obtained. To produce biogas, it is also possible to directly use the remaining solids from pectin extraction, supplemented with salts, producing 89.4 L/kg OPW (measured under normal conditions, 0 °C, 1 atm), with a CH_4 content of 66.7% (Ortiz-Sanchez et al., 2021). In this study, the experimental results were used as input data to simulate the biorefinery at different scales and with the economic analysis of the overall process. The authors concluded that the implementation of integrated biorefinery systems is only feasible at a small scale (Ortiz-Sanchez et al., 2021). In another similar study, Ortiz-Sanchez et al. (2020) found that the biogas yield obtained was 0.256 Nm^3/kg of OPW with a methane content of 45.7%. This fact highlights the difficulty to compare results reported in the literature.

Manhongo et al. (2021b) studied the process feasibility, economic viability and environmental impacts for Life Cycle Analysis of model integrated biorefinery of mango processing waste using three scenarios: 1) production of biogas; 2) co-production of pectin and biogas; 3) sequential recovery of pectin and polyphenols coupled with combined biogas and electricity production. Scenario 1 is the least attractive in terms of cost-effectiveness, with a net present value of -$86.4 million, compared to $50.0 million and $57.2 million for Scenarios 2 and 3, respectively. However, the results of the life cycle analysis suggest that Scenario 1 is the best in terms of global warming potential (GWP) of 12.1 kg CO_2 eq/ton of waste processed, compared to Scenarios 2 and 3, with GWP values of 12.8 and 15.9 kg CO_2 eq/ton, respectively. Regardless, the theoretical results demonstrated the economic and process feasibility of the co-production of bioenergy and bioactive compounds, and that it is necessary to balance environmental and economic benefits when making decisions for the implementation of agri-industrial waste biorefineries. To make the anaerobic digestion process more cost-effective, another possibility is to attempt to increase the yield of biogas production. Many types of waste are recalcitrant to this process, such as lignocellulose, with large polymeric molecules that are not directly accessible to methanogenic microorganisms. Physical and chemical pre-treatment methods have been utilised to some extent on an industrial scale for the delignification of waste to enhance biomethane production. However, these methods are energy intensive, expensive and not environmentally safe, and have the ability to generate toxic compounds including carboxylic acids, furans and phenolic compounds which may be inhibit methanogenic activity (Usmani et al., 2021). In comparison with other methods, biological pre-treatment offers more techno-economic advantages (Kamusoko et al., 2019). In this line, Hansen et al. (2021) report that the hyperthermophilic anaerobic bacterium, *Caldicellulosiruptor bescii*, is effective in degrading and solubilising lignocellulosic materials, producing sugars, acetate, lactate and lignin fibres. This pre-treatment substantially improves biogas yields by increasing the processing rate of lignocellulosic waste. Enzymatic biocatalysis of renewable lignocellulosic biomass is a key solution for the circular economy and mitigation of growing environmental impacts. To enhance biogas production, the application of nanomaterials has been studied. Among the most promising is zero-valent iron. The growth of the microorganisms beneficial to the AD process and the enzymes involved is stimulated and AD stabilised in the presence of this type of nanomaterials (Dehhaghi et al., 2019).

Another possibility for gas production is by physical processes from SDW. After the extraction of essential oils and pectin from OPW, Dávila et al. (2015) developed a process to generate electricity from the remaining solids (composed of cellulose, hemicellulose and lignin) using a gas turbine after gasification at 850 °C. Finally, the gases are cooled to 120 °C. In this process, from 1 ton/h of orange peel, 9.2 and 42.6 kg/h of *p*-cymene and pectin were obtained with purities of 97 and 81%, respectively, and 99.8 kWh of electricity. However, environmental and techno-economic analyses showed that the most suitable scheme was without electricity generation, reaching a production cost of 5.3 and 3.5 USD/kg *p*-cymene and pectin respectively.

4.1.3. Solid biofuel

Solid biofuel production is another alternative explored in biorefinery approaches. Adiletta et al. (2020) and Brachi et al. (2017) studied the valorisation of SBP and proposed that the pectin-free solid (about 75% of the original feedstock) could be torrefacted (250 °C, 30 min) obtaining a fuel with mass and energy yields of 48.1% and 66.1%, respectively. In addition, the calorific value of the depectinised SBP after torrefaction was increased from 16.7 MJ/kg DS to 22.5 MJ/kg DS. The solid fuel obtained was of high quality due to the low nitrogen and ash content. Moreover, the condensable fraction could be a source of valuable chemicals (e.g. D-limonene, furfural, levoglucosan) (Brachi et al., 2017). In a possible small-scale use, Vukušić et al. (2020) obtained pellets from the remaining SDW from apple pomace, with characteristics very similar to wood pellets (a net calorific value of 20.3 MJ/kg). According to these authors, for 1 ton of wet apple pomace the amount of pectin recovered was 22 kg, 232 kg of pellets and 38 kg of concentrated pectin-free pomace extract, used as part of a substrate for microbial *S. cerevisiae* growth.

4.2. Physical and chemical transformation of agri-food by-products

Potentially interesting biomaterials have been formed using conventional methods. Yates et al. (2017) developed an innovative approach based on depectinised apple pomace, which could be used as a biocompatible scaffold. By heating at 500 °C, this solid was transformed into a material capable of acting as a scaffold for cell growth in hard and soft tissue engineering. Talekar et al. (2018b) elaborated another material of interest of the SDW from PPW. This was dried, ground and carbonised by pyrolysis (5 h, ramp 5 °C/min up to 1100 °C in a N_2 atmosphere) and, subsequently, impregnated with potassium hydroxide and heated in an inert atmosphere to obtain hard carbon to be used as an electrode in electrochemical cells (7% DS yield). Very recently, Torres-Sciancalepore et al. (2022) studied the thermodynamic behaviour of the pyrolysis of quince waste and pectin-free quince waste after the extraction of pectin with acid (pH 2 with HCl, 60 °C, 2 h), obtaining a low yield (3.2% DS). They found that the extraction pre-treatment produces a solid residue that is more reactive and easier to convert through pyrolysis (5–15 °C/min, up to 900 °C), allowing multiple products to be obtained from quince waste.

To prepare semi-crystalline biocomposites with poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) for food packaging applications, Vannini et al. (2021) developed a process from the waste of the industrial extraction of sweet potato starch, using the solid resulting after the extraction of proteins and pectins. This fraction, rich in starch and fibrous components, was added in various amounts, up to 40% (w/w), by melt mixing, at 200 °C for 5 min or at 180 °C for 6 min, to prepare the biocomposites. In order to obtain other biocomposite-based materials, Zannini et al. (2021) fortified pectin extracted from CW with different amounts of the lignocellulosic fraction (15–35%) recovered from the SDW, following a "zero waste" circular economy approach. The prepared biocomposites were morphologically and mechanically characterised for use as a biodegradable mulching system for crop protection, showing an increase in the resistance to breaking when the lignocellulose residue (filler) was added in an amount equal to 25% with respect to the pectin content.

In a more traditional approach, cellulose and lignin have been obtained by alkaline hydrolysis from different depectinised materials. Ma et al. (2019) extracted pectin and cellulose from apple pomace waste. After pectin isolation, 90% of the lignin was removed at 70 °C, pH 4.0, 6.0% $NaClO_2$, for 2 h and 20% solids. To remove the hemicellulose, the solid (15% w/v) was then treated with 10% NaOH at 70 °C for 4 h, leaving a cellulose-enriched solid. In a pilot scale test (1000 g of apple pomace DS), 196 g of pectin and 244 g of cellulose (90.4% purity) were obtained. Previously, Szymańska-Chargot et al. (2017) demonstrated the potential of the sequential extraction of polyphenols, pectin and cellulose from carrot, tomato, cucumber and apple pomace. From the depectinised solids, hemicellulose was obtained with alkaline solution (1 M NaOH, 30 min, 85 °C), repeated 3 times, obtaining a yield between 4.3 and 5.7% for cucumber and carrot, respectively. An oxidative reagent, $NaClO_2$ (1–2%, 90 °C, 60 min, 2 times) was used to remove lignin (carrot 2.5%, tomato 5.9%) and leave cellulose (apple 17.2% and cucumber 25.0%). However, although this combination of chemical extraction methods was very efficient, the overall process should be optimised and simplified, as this would not be cost-effective. Similarly, by alkaline hydrolysis (pH 13, 125 °C, 1 h) and subsequent drying, Shinde et al. (2020) obtained lignin from PPW (yield 13%) after sequential extraction of ellagic acid and pectin. The advantage of sequential extraction is that it improves the economic viability of the process and reduces the total greenhouse gas emissions of each product. Pereira et al. (2021) studied the recovery of carbohydrates from banana peel by sequential treatment (acid, alkaline, and enzymatic). Pectin was extracted (8% yield) with the first pre-treatment, with 35% of xylose and xylo-oligosaccharides (XOS) from hemicellulose remaining in the liquid fraction. The solid fraction was then subjected to alkali process to remove lignin (31.6% of initial mass) and, finally, other fraction, subjected to enzymatic reaction could be used to produce bioethanol. After pectin extraction (yield 24.6%) and delignification, Jahn et al. (2020) produced hemicellulose from SBP (yield 34.8%) by an alkaline process (140 °C, NaOH 2%). This fraction was tested as a flame retardant and flotation agent with satisfactory results, although further research is needed.

Arora et al. (2018) evaluated the costs and profitability of a biorefinery plant for mango processing waste (10 tonnes/h) using conventional methods. They evaluated three possible alternatives, 1) recovery of polyphenols and pectin, 2) recovery of polyphenols, pectin and seed oil, and 3) "whole biorefinery", which includes processing mango seeds to release protein, starch and oil. The second option proved to be the best alternative because the other compounds in the seeds do not report a high benefit. Sensitivity analysis showed that capacity, plant operating days and raw material composition were the most important factors influencing plant economics, as well as the selling price of the main product, i.e. pectin. However, due to the low price of the raw material, this factor did not significantly influence the economics of the plant and, although the authors referred to a "whole biorefinery", the residual polysaccharides (cellulose and hemicelluloses) were not used.

Another factor to consider is that, the environmental impact of traditional methods would be reduced by using more environmentally friendly processes, such as US, MW, enzymatic or combined treatment, with mild conditions. With alternative processes, Balu et al. (2012) developed a MW-assisted process to transform the SDW from orange into mesoporous cellulose, achieving an overall utilisation yield of about 60% based on DS. The textural characteristics of the cellulose improved with increasing MW temperature. Mango peels constitute an important and interesting agri-food waste. It is the second most consumed tropical fruit, after bananas, and processing residues (peel, kernel and seed) account for 35–60% of the weight of the fruit. For the overall utilisation of these by-products, Matharu et al. (2016) studied their conversion into pectin and porous cellulose by a MW-assisted acid-free hydrolytic treatment. To obtain porous cellulose, the SDW was subjected to two-stage MW heating (600 W, 180 °C, 10 min 5% solid) and washed with ethanol and acetone. The residual cellulosic material exhibited mesoporous characteristics with an average pore diameter of about 10 nm and, after the second MW treatment, significantly increased in surface area, from 88.8 to 124.0 m^2/g and pore volume by about six times. Another simple and direct method has been developed by Valladares-Diestra, Porto de Souza Vandenberghe, et al. (2022) using a citric acid-assisted hydrothermal pre-treatment of cocoa pod husks for pectin recovery with concomitant production of XOS and subsequent efficient enzymatic hydrolysis of the SDW fraction to release monosaccharides. Under optimal conditions (120 °C, 10 min, solids 2% w/v) the recovery was 19.3% pectin, 5.2% XOS (recovered from LDW) and fermentable sugars 12.6% and 5.5% of glucose and xylose, respectively, leading to an

overall process yield of 42.6% DS. In a later work, the hydrolysed SDW was fermented with *S. cerevisiae* and LDW by *Candida tropicalis*, yielding 7.6% (w/w) of bioethanol respect to the original waste (Valladares-Diestra, de Souza Vandenberghe, & Soccol, 2022).

Some agri-food waste, such as green tea leaf after pectin extraction, can be used as a source of protein, which can be extracted conventionally using KOH (0.1 M, 95 °C, 3 h), yielding 230 g/kg protein and 370 g/kg solid for combustion (Zhang, Slegers, et al., 2018). Moreover, environmental benefits are also obtained by replacing conventional potassium (K) fertiliser with the wastewater from the K-rich protein extraction process.

4.3. Biotechnologically assisted valorisation of agri-food by-products

Pectin extraction residues and pectinaceous materials can be raw materials of great interest to the biotechnology industry as renewable sources to produce value-added products with environmentally friendly strategies, saving and reusing resources (Martins et al., 2020). In order to make the best use of valuable agri-food waste, intensive research has been carried out to identify efficient protocols to convert relatively low-value but highly abundant compounds into high-value products. In most cases, microbial fermentation plays a key role in these processes, although there are some interesting examples where utilisation does not occur by fermentation. This is the case of Cardenas-Fernández et al. (2017) and Cardenas-Fernández et al. (2018) who developed an integrated biorefinery process for SBP subjected to a steam explosion (152 °C, 5 bar, 24 min) separating a relatively pure cellulose solid and a liquid fraction, with monosaccharides and polysaccharides rich in arabinose and GalA. To release all the arabinose content, the liquid fraction was subjected to a continuous hydrolysis process with an immobilised α-L-arabinofuranosidase (3 g immobilised enzyme, 3.9 g/L substrate concentration, 5 h^{-1} dilution rate, 50 °C, 173 h effective process). The arabinose was separated by tangential flow ultrafiltration (UF, 1 kDa) reaching 90% recovery and 95% purity, and GalA-rich oligo- and polysaccharides were present in the retentate (Cardenas-Fernández et al., 2018). Senit et al. (2019) developed two uses of OPW through Taguchi's design of experiments after obtaining essential oils and phenolic compounds. One possibility was to obtain pectin (19.6% DS). Another, to increase the overall yield of the process, was to carry out enzymatic saccharification of all the material with a mixture of cellulases, β-glucosidases, xylanases and pectinases, which yielded GalA (250 g/kg DS), glucose (296 g/kg DS) and fructose and galactose (168 g/kg DS), achieving 71% DS yield in monomers. This post-treatment left low amounts of solid residues.

In addition, the biotechnologically-assisted valorisation of natural polysaccharides can provide a source for renewable fuels and chemicals by enzymatic reactions. Early studies related to the conversion of polysaccharides to fermentable sugars focused on glycoside hydrolases. However, in recent years it has been an increase in the use of lytic polysaccharide monooxygenase (LPMOs) that are able to break the strong C–H bonds in the highly recalcitrant polysaccharide chains via an oxidative mechanism (Meier et al., 2018). These enzymes oxidise the C1 or C4 of the sugar units of polysaccharides, weakening the glycosidic bond. Improving enzymatic saccharification of lignocellulosic biomass is the major bottle-neck in the area of second generation biofuel production. Recently, Sepulchro et al. (2021) showed that photocatalysis could significantly increase the LPMO activity against highly crystalline and recalcitrant cellulosic substrates, which are poorly degraded in the absence of chlorophyllin and light. This enzyme could be applied to the production of cellulose-derived nanomaterial building blocks, including cellulose nanoparticles, nanofibrils and nanocrystals.

4.3.1. Fermentation-assisted valorisation

The main high value-added products obtained by fermentation of agri-industrial waste are organic acids (succinic, citric, lactic, among others), bioplastics, dyes, enzymes and other platform chemicals. Of these, the organic acids have the largest niche market but the lowest price, as they are used as platform ingredients in many applications (including bioplastics) (Teigiserova et al., 2019). Fermentation processes are highly suitable for the production of organic acids from agri-food waste, as they provide substrates with a high moisture content. In general, feedstock with a moisture content of between 60 and 85% is favourable for bacterial growth, while fungi require a lower moisture content of between 40 and 60% (Teigiserova et al., 2019).

One of the acids whose production from depectinised substrates has been studied is succinic acid. Patsalou et al. (2017; 2020) have studied its production by fermentation with *A. succinogenes* from CW. Patsalou et al. (2017) followed two approaches, using the solid remains from pectin extraction and the liquids from pectin isolation as the starting material by direct fermentation of the releasing sugars (glucose equivalents 0.21 g/g DS), achieving a succinic acid yield of 0.77 g/g total sugar consumed, equivalent to 6.13 g/L. In a second trial, the solid was treated with cellulases and β-glucosidases (30 U/g and 25 U/g, respectively; 48 h at 50 °C) releasing monosaccharides, 0.58 g/g DS, which were fermented producing a succinic acid yield of 0.7 g/g of total sugar consumed, corresponding to 8.3 g/L. However, although the enzymatic treatment increased the succinic acid yield by 26%, the total cost of production indicated that the use of enzymatic hydrolysis could not be competitive. Taking into account these results, Patsalou et al. (2020) developed the process at a pilot plant scale without prior saccharification, by the supplementation of the hydrolysate with maize liquor and vitamins. Batch-fed fermentation was then performed, obtaining 22.4 g/L succinic acid from 54 g/L of initial total sugars. These authors also used the remaining biorefinery residues as a fertiliser substitute in an attempt to substantially reduce the process residues. Using the same fermentation procedure, Alexandri et al. (2019) obtained succinic acid from depectinised SBP. In this case, cellulose and hemicellulose, present in the solid residue, were hydrolysed by acid pre-treatment (30 min with 0.5% H_2SO_4 at 121 °C), and subsequent enzymatic hydrolysis with cellulase. A conversion yield of 55% hemicellulose and 36% cellulose at pilot scale was achieved, yielding 44 g/L of total sugars (initial concentration 10% solids) with glucose and arabinose (19 and 16 g/L, respectively) as main monosaccharides. Finally, fed-batch fermentations of SBP hydrolysate with *A. succinogenes* were carried out, resulting in a succinic acid production of 268 g/kg DS of SBP.

From SBP it is also possible to obtain lactic acid after extraction of pectin (Alves de Oliveira et al., 2020). This material was composed of 31.6% hemicellulose, 21.5% cellulose, 9.7% protein, 8.7% free sugars and 8.5% ash as main components. Before fermentation, the purified SBP (10% w/w) was pre-treated at 121 °C for 30 min with 0.5% (v/v) H_2SO_4. Subsequently, the pH was adjusted to 5 and hydrolysed with cellulase, pectinase and protease (50 °C, 24 h), yielding an average sugar concentration of 16.5 g/L glucose, 9.6 g/L xylose and 10.0 g/L arabinose. The batch fermentation with *Bacillus coagulans* was carried out at 52 °C, 400 rpm, pH 6; after 150 h, all sugars were consumed, obtaining a concentration of 0.71 g of lactic acid/g of sugars. Considering this fermentation, an assumed biorefinery process of about 100,000 tonnes/year of SBP could produce 20,000 tonnes/year of lactic acid, 7600 tonnes/year of phenolic compounds, 29,200 tonnes/year of pectin, and 20,000 tonnes/year of animal feed with 20% protein content.

Based on the production of lactic and succinic acid from fermentative processes of renewable resources such as glucose syrup, corn stover and SBP, Ioannidou et al. (2022) provide a techno-economic evaluation, life cycle assessment and life cycle costing for the production of poly (butylene succinate) and poly (lactic acid). These biopolymers are among the most widely used, with an annual global production capacity of 86,500 tonnes and 394,500 tonnes. In the SBP-based biorefinery process, the separation of pectin as a co-product is considered. Process analysis indicates that the potentials of acidification, eutrophication and human toxicity were lower when SBP is used. The techno-economic risk assessment, carried out using Monte-Carlo simulations, showed that the development of biorefinery based on depectinised SBP ensures a more

sustainable production of poly (lactic acid) and poly (butylene succinate) compared to their fossil-based counterparts and to bioprocesses using glucose syrup and corn stover.

Butyric acid can also be obtained from the depectinised residues. In the process developed by Wang et al. (2021), PPW was delignified (30 mM phosphotungstic acid, γ-valerolactone as a sustainable solvent at 130 °C for 3 h) and the paste was washed and dried. Cellulase was then added to the solid (1000 U/mL, 3% DS, 30 °C, pH 5.2, 72 h) and an elevated amount of glucose was released (93.3% of the total). Finally, these fermentable sugars were used as a carbon source for the production of butyric acid using *Clostridium tyrobutyricum*, obtaining yields of 0.44 g/g glucose, very similar to the yield achieved with glucose alone at 0.46 g/g.

Theoretically, mucic acid can be produced from OPW by fermentation with *Trichoderma reesei* (pH 4, 35 °C) of the liquid fraction of the pectin extraction, as a source of GalA, enriched with lactose and yeast extract, obtaining a yield of just 2%, although it is a high-value product. In this simulation, once again, the authors highlight the low influence of raw material costs on the economic viability of the process. Finally, the environmental analysis showed that the impact of the output streams is lower than the impact of the feed streams (Ortiz-Sanchez et al., 2020). In this approach, biogas production (8.8%) was also considered.

Other compounds synthesised by yeasts are fruit-type volatile aromatic compounds, single-cell oil and lipids, single-cell proteins, carotenoids and enzymes. The following are some examples of interest of value-added products obtained from pectin-rich residues. Kazemi et al. (2019) obtained pullulan after recovery of pectin and phenolic compounds from eggplant waste. The remaining solids were enzymatically hydrolysed with cellulase and the hydrolysates were used as a carbon source in the microbial production of pullulan by fermentation with *Aureobasidium pullulans*. The yield obtained was 16.8 g/L (0.56 g/g sugars consumed) and this pullulan showed similar chemical characteristics to commercial pullulan with α-(1–6) and α-(1–4) glycosidic linkages, and ash and protein content of 1.8% and 2.1%, respectively.

To produce bacterial cellulose, the pectin-free solid residue of the OPW was subjected to hydrolysis of hemicellulose (72.5%) and cellulose (70.4%) by sequential pre-treatment with dilute H_2SO_4 and enzymatic hydrolysis, using the commercial enzymes Viscozyme® L and β-glucosidase from almonds. Sugars released after hydrolysis and free sugars, extracted at the beginning of OPW processing, were utilised by *Komagataeibacter sucrofermentans* in tray bioreactors under air sparging, leading to a bacterial cellulose productivity of 14.2 g/kg DS (Tsouko et al., 2020). In another study, Karanicola et al. (2021) used dilute acid hydrolysis in a US bath (40 kHz) for the production of essential oils, pectin and bacterial cellulose from OPW. The optimum conditions, obtained using a desirability function, were 5.75% solids, 1.21% H_2SO_4 and 34.2 min. The yields obtained were 0.12% essential oils, 45% pectin and 40% hydrolysed sugars, at both laboratory and pilot scale. This hydrolysate was fermented by *K. sucrofermentans,* producing 5.8% on DS of bacterial cellulose. The *Komagataeibacter* genus is the best producer of cellulose using agricultural waste. The main advantages of bacterial cellulose are the nanoporous structure, high water content and free hydroxyl groups (Cacicedo et al., 2016).

4.4. Residual biomass uses

In many biorefinery processes, biomass is produced as a final waste form that could also be used, the composition of which varies greatly depending on the previous processing of the agri-food waste and its intended use. In a more unspecific way, the uses of this biomass are usually animal feed or agronomic use for soil improvement. An initial type of residual biomass that can be considered is SDW, whose direct use can be as livestock feed. Fidalgo et al. (2016) described this use for the organic residue obtained using water and MW for the extraction of pectin and essential oils from fresh lemon peel, which was mainly composed of hemicellulose and cellulose. Similarly, SDW from rapeseed cake treated with enzymes can be used as protein-rich feed (Jeong et al., 2013). Other type of residual biomass is the remaining material after saccharification and/or fermentation. A good example is SBP, a protein-rich by-product that can be used directly as animal feed for ruminants due to its high cellulose content, after extraction of pectin (Zema et al., 2018), after saccharification (Alexandri et al., 2019; Alves de Oliveira et al., 2020) or after fermentation (Hamley-Bennett et al., 2016). The final insoluble product, enriched in protein (20% DS) and with low fibre content, into which the cells produced during fermentation can be incorporated, can be used as feed for poultry and other animals, not necessarily ruminants. Another study reports that *M. indicus* was not suitable for bioethanol production. However, its growth on SDW from citrus for 24 h resulted in fungal biomass yields of 454 mg/g sugars consumed, increasing up to 687 mg/g sugars consumed after 72 h. Furthermore, this biomass was enriched in polyunsaturated fatty acids and protein and was a nutritionally valuable food (Satari et al., 2017).

Zema et al. (2018) compiled literature information on the direct use of CW for agronomic utilisation, indicating that if sufficient agricultural land is available near processing industries, CW can be used as an organic soil conditioner or as a substrate for compost production. This use is also possible for the DSW and for the biomass remaining after a fermentative process. Patsalou et al. (2017; 2020) highlighted that, after fermentation with *A. succinogenes*, the remaining residues can be used as a fertiliser substitute in an attempt to substantially reduce processing residues.

5. Associations between by-product composition and high-value ingredients recovered: a comparative study

Numerous studies have reported a wide range of valorisation strategies of all fractions resulting from pectin extraction. However, these applications might be determined by the structural characteristics of the source material. It should be noted that several studies summarised in Table 2 report obtaining several fractions from the same agri-food by-product. For example, Gonzalez-Garcia et al. (2018) obtained two different fractions from beet by-products following hydrothermal or enzymatic treatments, while Encalada, Pérez, Calderón, et al. (2019) isolated different fractions from carrot by-products using ultrasound or alkaline treatments. As can be shown, these fractions led to different yields of functional ingredients. Therefore, these fractions have been considered as different samples to perform statistical analyses. To establish associations between by-product composition and the recovery of pectin and other functional ingredients in a biorefinery context, a principal components analysis (PCA) of composition parameters (cellulose, hemicellulose, lignin and protein content) was first performed (Fig. 4). Sample distribution was studied according to three different criteria: 1) by-product source, 2) pectin extraction method used, 3) microbial species selected in those studies reporting microbial fermentation.

By-product source. Beet by-products, showing high hemicellulose and low lignin content, presented a completely different profile from those by-products from apple, banana, mango and pomegranate (Fig. 4A). In addition, rapeseed by-products rich in cellulose were greatly discriminated from carrot, eggplant and melon waste. A great variability in the composition of citrus by-products was observed.

Pectin extraction method. As can be seen in Fig. 4B, there is no clear relationship between by-product composition and the pectin extraction method selected. This fact indicates that these pectin extraction techniques have been applied to numerous by-products showing very different composition profiles, highlighting the wide range of applications of these methods. In general, substrates showing low cellulose and high protein content were selected for pectin extraction using organic acids and enzyme preparations (Fig. 4B). In contrast, substrates subjected to US-assisted extraction generally showed low carbohydrate or protein content. Future studies will aim at determining the suitability of conventional and novel pectin extraction methods depending on the

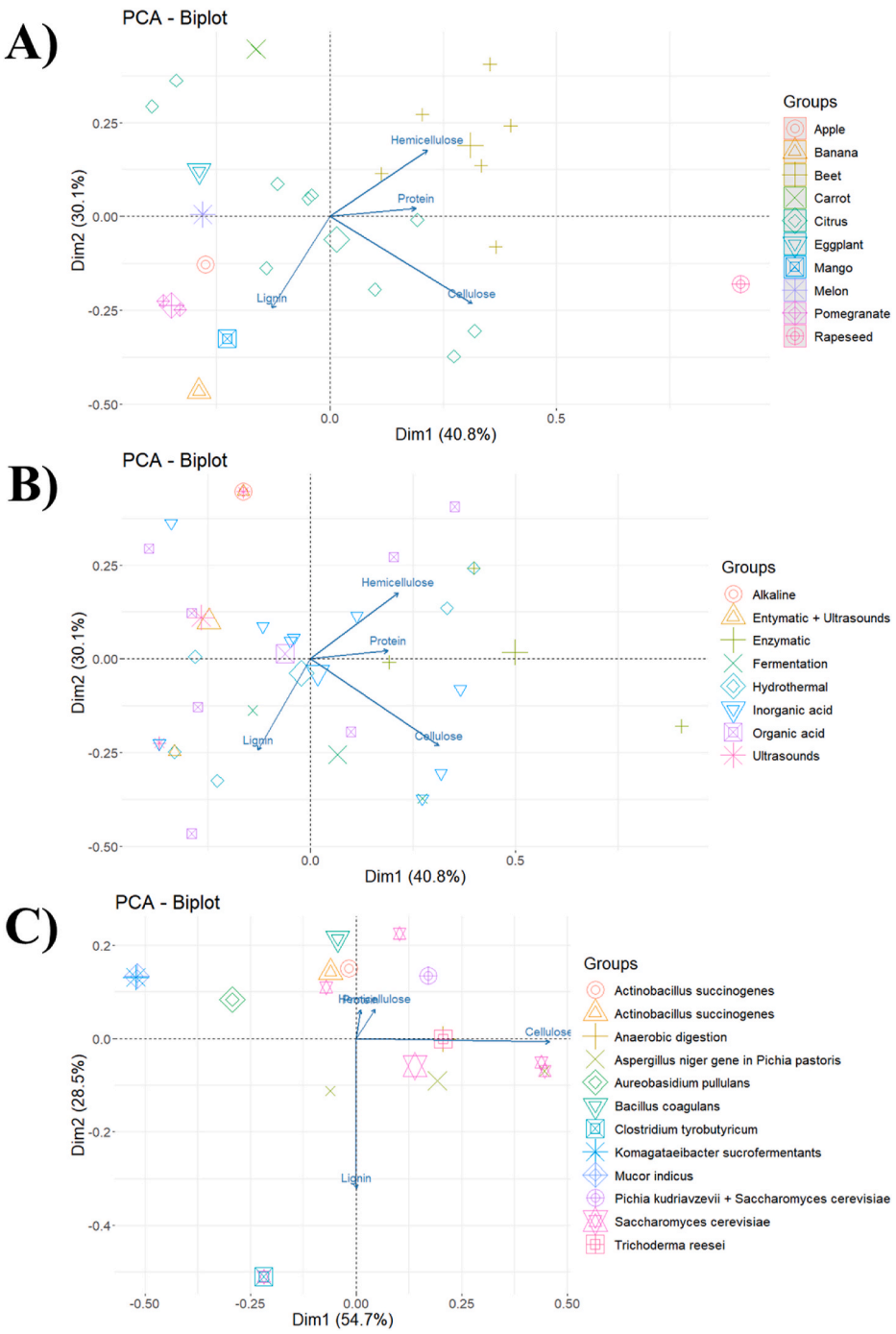

Fig. 4. Principal components analysis (PCA) of fruit and vegetable by-product composition (expressed as cellulose, hemicellulose, lignin and protein contents) according to by-product source (**A**), pectin extraction method used (**B**), and microbial species selected in those studies reporting microbial fermentation (**C**). Dim: dimension (principal component).

carbohydrate composition of fruit and vegetable by-products. Carbohydrate characterisation of a wider range of by-products may allow pectin yield to be maximised and may simplify the integrated biorefinery process.

Microbial species. Most by-products subjected to microbial fermentation showed low lignin content, with the exception of those fermented by *C. tyrobutyricum* (Fig. 4C). In addition, substrates fermented by *S. cerevisiae* were rich in cellulose contrary to those fermented by *A. pullulans* and *K. sucrofermentans*. However, the number of studies reporting the applications of these species, in the context of this review, is limited and relationships described here may provide an initial insight into the fermentative properties of pectin-rich by-products.

Once composition profiles of different fruit and vegetable by-products have been described (Table 2), associations between by-product composition and high-value ingredients recovered during the biorefinery process were investigated. For this purpose, Pearson correlation coefficients were calculated. Strong negative and moderate positive associations between the degree of methyl-esterification of pectin and hemicellulose and cellulose content of by-products, respectively, were found. It should be noted that vegetable by-product fractions showed higher hemicellulose content that those isolated from fruit wastes (Table 2). It has been reported that pectin from vegetables

showed a lower degree of esterification than that obtained from fruits (Encalada et al., 2019b). A moderate positive correlation between GalA content and lignin content of original by-products can be explained because pectin may be more easily separated from lignin than other polysaccharides, leading to a higher pectin purity (expressed as GalA content).

With regard to other high-value ingredients obtained in the biorefinery process, essential oil yield showed a strong positive correlation to all composition parameters. Essential oils are isolated from CWs that show high cellulose content and great variability in the rest of the parameters (Table 2, Fig. 4A). In contrast, the phenolic compound yield showed strong and moderate negative correlations to protein and hemicellulose content, respectively. Finally, biofuel and biogas yields showed a strong negative correlation to lignin content, and strong positive correlations to hemicellulose and protein content. These correlations highlight that high biogas yields are obtained from the fermentation of by-products with high hemicellulose content (Table 2). It has been reported that agri-food by-products showing low lignin content are interesting substrates for biofuel production (Martins et al., 2020).

In general, a more in-depth study of these correlations will allow the development of more efficient processes in which an integral use of the different types of waste is achieved depending on their initial composition and the sequential extraction processes. Finally, it seems to be of great interest to study glycosidase activity from microbial species used in biorefinery approaches involving fermentation processes (Table 2). Protein sequences of reference genomes from *A. succinogenes*, *A. niger*, *A. pullulans*, *B. coagulans*, *C. tyrobutyricum*, *K. sucrofermentans*, *P. kudriavzevii*, *S. cerevisiae* and *T. reseei* were retrieved from the National Center for Biotechnology Information (NCBI) database (National Center, 2022). These sequences were then annotated following the "run_dbcan" pipeline developed by Zhang, Yohe, et al. (2018), which maps the samples against the Carbohydrate-Active enZYmes Database (CAED, 2022). To ensure the quality of glycosidase identification, only glycosidase domains showing coverage values higher than 0.95 were selected for further analysis.

A heatmap illustrating the presence and absence of specific CAZy domains in reference sequences from these microbial species is provided in Fig. 5. As can be seen, *A. pullulans*, *A. niger* and *T. reesei* showed the widest range of pectin-hydrolysing glycosidase domains. As a consequence, these three species may be of special interest to obtain modified pectin and POS derived from pectin depolymerisation. In fact, Yang et al. (2020) reported the incorporation of *A. niger endo*-polygalacturonase genes in *P. pastoris* to obtain POS from CW fermentation. Similarly, *A. pullulans*, *A. niger* and *T. reesei* were the only species showing glycosidase domains involving alcohol oxidases. In addition, these species (*A. pullulans*, *A. niger* and *T. reesei*) could provide complementary glycosidase activity to "non-conventional" yeasts like *Kluyveromyces marxianus* and *Meyerozyma guilliermondii,* that can natively assimilate pectic sugars in order to produce bioethanol, unlike wild *S. cerevisiae* strains (Sharma et al., 2021).

Bioethanol production from non-edible feedstocks rich in lignocellulosic materials has been widely studied (Panahi et al., 2022). However, fewer studies have reported microbial fermentation for bioethanol generation from pectin-rich matrices and are very scarce for pectin extraction side-streams (Table 2). In this sense, co-culture simultaneous fermentation of sugarcane bagasse with an ethanologenic *E. coli* in combination with *S. cerevisiae* has been used to obtain bioethanol (Wang et al., 2019). However, no relevant pectin-active domains could be annotated in the *S. cerevisiae* genome in the present study. This fact may indicate that *A. niger*, *A. pullulans* and *T. reesei* may be better suited to the bioconversion of pectin-rich matrices than other species that have been broadly used to valorise other lignocellulosic substrates (Panahi et al., 2022). It should be noted that these microorganisms are suitable for fermentation processes other than ethanolic fermentation.

Previous studies have shown that alcohol oxidase enzymes for biogas production from lignocellulosic substrates are of interest (Hernández-Beltrán et al., 2019; Wei, 2016; Čater et al., 2014). Fermentation of fruit and vegetable by-products using *A. pullulans* to obtain pullulans (Kazemi et al., 2019), and biogas (Ortiz-Sanchez et al.,

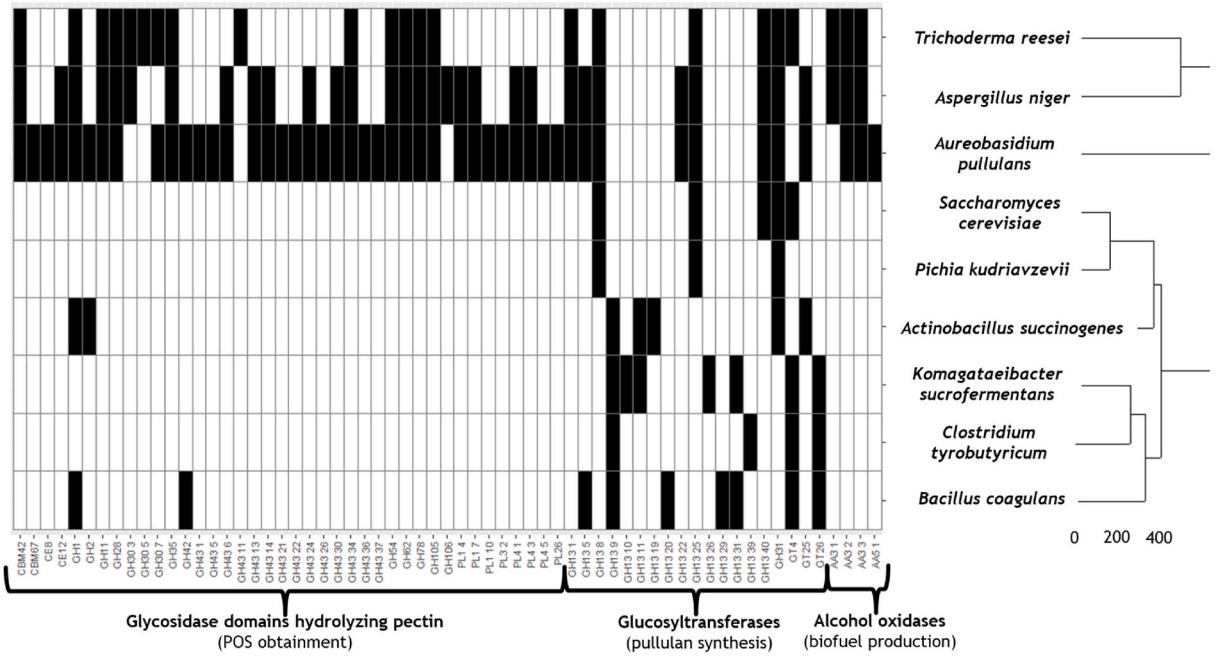

Fig. 5. Heatmap showing the presence of different glycosidases (indicated as black cells) in the reference sequence proteins of several microbial species used in fermentation processes for the valorisation of fruit and vegetable by-products. Specifically, functional domains involving glycosidases capable of degrading pectin to yield pectic oligosaccharides (POS), glucosyltransferases participating in pullulan synthesis and alcohol oxidases that may be of interest for biofuel production are illustrated. Glycosidase functional domains showing coverage values higher than 0.95 were annotated. Codes corresponding to the CAZy family of each enzyme have been assigned. More information about these families can be found in the Carbohydrate-Active enZYmes Database (CAZy).

2020), was also reported. In this sense, the *A. pullulans* protein sequence showed seven functional domains containing glucosyltransferases that may be involved in pullulan synthesis, in agreement with the experimental process described by Kazemi et al. (2019). Other species that may be of particular interest for pullulan production may be *A. niger*, *B. coagulans* and *K. sucrofermentants* due to the presence of glucosyltransferase domains in their protein sequences. Based on the results obtained, it seems of great interest to carry out a preliminary study of the glycosidase activity of a microorganism before using it in a biorefinery process, so that the optimal use of each by-product can be predicted according to the metabolite to be produced.

6. Conclusions and future trends

This review provides a general overview of pectin extraction from fruit and vegetable by-products in a biorefinery approach. In addition, obtaining high-value compounds from each fraction is described. Valorisation of fruit and vegetable by-products mitigates their negative environmental impact and may lead to a transition toward a bioeconomy model. A systematic review of the current literature available in this field revealed the importance of consolidated research. Waste biomass valorisation and optimisation of enzymatic processes for biorefinery platforms have attracted a great deal of attention. For this purpose, the utilisation of commercial preparations, microbial fermentation, and anaerobic digestion has been reported. Improving the enzymatic saccharification of lignocellulosic biomass is the main bottleneck in the field of second-generation biofuel production, and the use of combined glycosidase and LPMO enzymes could favour the complete hydrolysis of polysaccharides. In terms of substrates, CW is the most widely studied. However, in recent years, these methodologies have also been applied to other substrates that show great variability in their composition. The scientific literature highlights the suitability of biotechnological processes to achieve high compound yield and purity, and the number of papers reporting obtaining functional ingredients from pectin-rich substrates has increased in recent years. These compounds comprise pectin and POS, with different structural features, that may result in different biological activity, phenolic compounds and essential oils. The production of other polysaccharides like cellulose and pullulan, as well as organic acids and microbial biomass, is also reported. These ingredients could be of special interest for functional food formulation and may provide an additional value compared to traditional approaches focused on bioenergy production.

Composition profiles of fruit and vegetable by-products reported in the bibliography and experimental yields of high-value ingredients have been compared in this review as an initial approach to assess their valorisation potential. The number of studies currently available is limited. Therefore, future research will aim at covering a wider range of substrates in order to develop more efficient processes. This strategy will lead to the integral use of different types of waste depending on their overall composition with a strong emphasis on carbohydrates. Future studies may also join in knowledge of microbial metabolism to optimise the use of each by-product and to design more selective green processes based on those metabolites potentially produced. In the present study, it was found that *A. pullulans*, *A. niger* and *T. reesei* showed a high number of pectin-active enzymes compared to other species reported in the literature. These preliminary results may provide a template for in-depth studies aiming at elucidating metabolic networks and cross-feeding interactions of these species. As a consequence, it could be possible to design specific microbial consortia for the production of functional ingredients from pectin-rich biomass.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Carlos Sabater: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Mar Villamiel:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Antonia Montilla:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare not conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.107564.

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