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Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids

Stanislav Obruca[1], Pavla Benesova[1,2], Dan Kucera[2], Sinisa Petrik[1], Ivana Marova[1,2]

[1]*Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic*

[2]*Department of Food Science and Technology, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic*

**Corresponding author: Stanislav Obruca, Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkynova 118, 612 00 Brno, Czech Republic. E-mail: Stana.O@seznam.cz, Tel: +420 541 149 486, Fax: +420 541 211 697*

Abstract

Coffee is one of the world´s most popular beverages and has been growing steadily in commercial importance. Nowadays, coffee is the second largest traded commodity in the world, after petroleum. Hence, coffee industry is responsible for the generation of large amounts of waste, especially spent coffee grounds (SCG). Various attempts to valorise this waste stream of coffee industry were made. This article summarizes our research and publications aiming at the conversion of SCG into valuable products – polyhydroxyalkanoates (PHAs) and carotenoids. At first, oil extracted from SCG (approx.15 wt % oil in SCG) can be efficiently ($Y_{P/S}$ = 0.82 g/g) converted into PHA employing *Cupriavidus necator* H16. Further, the solid residues after oil extraction can be hydrolysed (by the combination of chemical and enzymatic hydrolysis) yielding fermentable sugars, which can be further used as a substrate for the production of PHAs employing *Bacillus megaterium* ($Y_{P/S}$ = 0.04 g/g) or *Burkholderia cepacia* ($Y_{P/S}$ = 0.24 g/g). Alternatively, SCG hydrolysate can be used as a substrate for biotechnological production of carotenoids by carotenogenic yeast *Sporobolomyces roseus*. Solid residues after either oil extraction or hydrolysis can be used as fuel in industrial boilers

to generate heat and energy. Therefore, entire biomass of SCG can be used for sustainable production of PHAs and/or carotenoids employing bio-refinery approach.

Key words: Spent coffee grounds, polyhydroxyalkanoates, carotenoids, bio-refinery

Coffee industry and the production of spent coffee grounds

Coffee represents very popular beverage which has been consumed for over 1000 years and today it is considered as the drink consumed the most throughout the world (more than 400 billion cups per year) [1]. Hence, coffee has been growing steadily in commercial importance during the last 150 years and; nowadays, coffee is the second largest traded commodity in the world, after petroleum. In 2010, the worldwide annual production of coffee beans exceeded 8 million tons [2]. Coffee industry is, therefore, responsible for the production of large quantities of solid as well as liquid waste streams, especially spent coffee grounds (SCG). This solid residue is generated during the coffee beverage preparation or the soluble (instant) coffee manufacturing, when raw coffee powder is mixed with hot water or steam under the conditions which enable the aroma compounds and other coffee-bean constituents to be released into the liquid. SCG is a residue with fine particle size, high humidity (in the range of 50 - 85%) and organic load. It was stated that almost 50 % of the worldwide coffee production is used for instant coffee preparation [3]. Numerically, 1 ton of green coffee generates about 650 kg of SCG and about 2 kg of wet SCG are obtained from each 1 kg of instant coffee produced [1]. Therefore, SCG is generated in large amounts, for instance Tokimoto et al. estimated the worldwide annual generation of SCG to be 6 million tons [4]. Although SCG are available especially in coffee producing countries, Europe also belongs among the regions with strong coffee industry and high per capita consumption. In 2012, approx. 3.12 million tons of green coffee beans were imported to EU to be processed and manufactured and in the same year 355,777 tons of instant coffee was produced in EU (European Coffee Report 2012/2013). Since the manufacturing of 1 kg of instant coffee generates approximately 0.91 - 1.2 kg of SCG [6], SCG available just from coffee manufactories in EU countries make more than 330.000 tons. Furthermore, the important point is that SCG can also be relatively easily collected not only from instant coffee producing factories but also

from fast food chains, cafeterias, restaurants etc. Thus, Zuorro and Lavecchia estimated that almost 10,000 tons of SCG can be collected per year just in the Province of Rome [7].

The chemical composition of SCG is shown in Table 1. SCG contain major portion (30-40 wt. %) of hemicelluloses, in particular mannans, galactans with only minor portion of arabinans. On the other hand, the content of cellulose in SCG is only about 10 wt. %. Moreover, SCG contain significant amount (approx. 15 wt. %) of oil. Lignin is also very important constituent of SCG, which contributes to the fact that SCG possess high calorific value [2, 5]. Polyphenols of SCG are represented mainly by highly bioavailable and bioactive chlorogenic acids [6].

Due to the presence of organic materials SCG are highly polluting residues and require large quantities of oxygen to degrade. In addition, SCG reveal partially toxic nature due to the presence of caffeine, tannins, and polyphenols. Furthermore, simply piled up, SCG can ferment and combust spontaneously [7]. Therefore, SCG might represent a pollution hazard if discharged into the environment.

Potential strategies of SCG valorisation

Nowadays, great political and social pressure focuses on the reduction of pollution arising from industrial and agricultural activities. Consequently, most large companies no longer consider the residues as waste, but as a raw material for other processes. Targeting the field of agriculture and food industry, it is in agreement with recent concept of bio-refinery – the facility capable of maximal valorisation of biomass yielding high value products such as fuels, materials, chemicals and energy. Such an approach might reduce the dependence on petroleum which can be (at least partially) replaced by renewable or even original waste resources.

Hence, there is no surprise that SCG attract the attention of many researchers as a promising substrate for various processes enabling the conversion of SCG into value-added products. In Fig. 1 a rapid increase of number of published scientific articles dealing with spent coffee grounds after 2010 is demonstrated. It clearly shows that the valorisation of SCG has recently become an important research topic.

In some cases, SCG may be used as fuel in industrial boilers due to their high calorific power

of approx. 20-25 MJ/kg, which makes them comparable to other agro-industrial residues or soft-woods [7]. The possibility to use SCG as animal feed was also tested but high lignin content in this material was considered a limiting factor for this application [8] and it is also likely that the presence of polyphenols, caffeine and other substances in SCG probably restricts their application as animal feed as well. However, there are other technologies which might offer a strategy for the SCG valorisation, yielding high value products including biodiesel [5, 13, 14], ethanol [5, 16] or substances for pharmaceutical and cosmetic purposes [6, 9, 10, 11, 12, 15]. Table 2 provides brief summary of the processes which have been recently published in the literature suggesting possible strategies for the SCG valorisation.

Utilization of SCG for the production of polyhydroxyalkanoates

Apart from the processes mentioned above, SCG can also be used as a substrate for biotechnological production of polyhydroxyalkanoates (PHAs) – biodegradable and biocompatible polymers. PHAs are the family of hydroxyacid polyesters which are produced and accumulated in the form of intracellular granules by wide variety of bacterial strains which use PHA as carbon, energy and reducing power storage material. Due to their mechanical properties resembling polypropylene or polyethylene, PHAs are considered as an alternative to synthetic petrochemical-based polymers. Moreover, PHAs can be produced from renewable or even waste resources and, thanks to their biodegradability, they are environmentally friendly [24].

To compete with common plastics, the production costs of PHAs should be reduced. Because the price of media component contributes the most significantly (the carbon substrate up to 50%) to the overall production costs of PHAs, inexpensive or even waste substrates attract the attention of many researches [25]. Therefore, we decided to study the possible strategies to utilize SCG as a substrate for the PHAs production. This article presents the overview of our research and publications dedicated to the biotechnological conversion of SCG into PHAs.

At first we tested the suitability of oil extracted (using n-hexane) from SCG [27]. Generally, plant oils are very promising substrates for the PHAs production because the theoretical yield coefficient of PHAs production from oils is above 1 g of PHAs per g of plant oil. The fact is that oils

comprise higher number of carbon atoms per weight and, moreover, fatty acids are utilized via β-oxidation pathway directly yielding the precursor of PHB production – acetyl-CoA [28]. Moreover, oils which do not compete with human food chain and the utilization of which for the PHAs production results in the elimination of problematic waste (such as waste frying oils) are the most attractive substrates for the PHAs production [26]. Oil extracted from SCG meets all these requirements.

SCG oil exhibits relatively high acid value (caused by the presence of free fatty acids), which complicates its transesterification during the biodiesel production [13]. On the contrary, this property of coffee oil stimulated significantly the accumulation of homopolymer polyhydroxybutyrate (PHB) in *Cupriavidus necator* H16 when cultivated on SCG oil as the only carbon substrate. Hence, oil extracted from SCG revealed to be the best substrate for the PHB production among inexpensive/waste oils tested [27].

The cultivations were further performed in laboratory bioreactor using both, batch and fed-batch feeding strategies, the results are shown in Table 3. The utilization of coffee oil as substrate for the PHB production resulted in high productivity coefficients $Y_{P/S}$ 0.88 and 0.82 g of PHB per g of oil for batch and fed-batch cultivation, respectively [27]. Moreover, the fact, that the PHB production on coffee oil was accompanied by high PHB content within the biomass (90.1 and 89.1 wt. % for batch and fed-batch, respectively), has a positive impact not only on the productivity of the system, but it might also reduce the cost of the PHB isolation, because the PHB content in cells is an important factor influencing the economy of PHB down-stream processing [29].

The solid residues after the oil extraction can be used for the generation of heat and energy in industrial boilers, which should (at least partially) cover the energetic demands of oil extraction, bioreactor sterilization, fermentation and also polymer isolation [27]. On the other hand, we also investigated the possibility to hydrolyze delipidized SCG and to utilize the hydrolysate as substrate for the PHAs production. The hydrolysis of hemicelluloses was performed by the diluted acid treatment (1 % H_2SO_4; 90 min, 130°C) followed by the enzymatic cleavage of cellulose (cellulase Celluclast, Novozymes). The hydrolysis of 150 g of SCG per litre of solution yielded the hydrolysate (spent coffee grounds hydrolysate - SCGH) containing fermentable sugar at the concentration of 50.1 g/l.

The major sugars identified in SCGH were mannose and galactose followed by glucose, arabinose and cellobiose [30]. The fact that hexoses are substantially dominating sugars in SCGH may be an important factor positively influencing the production of PHAs and significantly reducing the problems related to the carbon catabolite repression [31].

We tested two different bacterial strains for their ability to produce PHAs on the SCG hydrolysate (SCGH) - *Bacillus megaterium* (unpublished data) and *Burholderia cepacia* [30]. Both strains were capable of SCGH utilization and PHAs production; however, *B. cepacia* revealed significantly higher PHAs yields and production coefficient (see Tab. 3). Moreover, *B. cepacia* was able to accumulate the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(HB-*co*-HV)], which has more desirable mechanical and technological properties than the PHB homopolymer produced by *B. megaterium*. Levulinic acid present in SCGH probably served as the precursor of 3-hydroxyvalerate for the copolymer biosynthesis by *B. cepacia* [30].

Apart from fermentable sugars, SCGH contained also a portion of potentially antimicrobial substances, mostly polyphenols (approx. 3 g/l). Hence, in order to improve the PHAs yields even more we tested various detoxification methods aiming at the removal of microbial inhibitors. Zuoro and Lavecchia reported that coffee phenolics can be extracted from SCG by the mixture of water and ethanol [6]; therefore several concentrations of ethanol were tested. Apart from the extraction of polyphenols prior to hydrolysis, also the post-hydrolysis detoxification methods such as the overliming and the application of activated charcoal were carried out. However, the extraction of polyphenols from SCG by 30 % ethanol prior to the hydrolysis seems to be the most efficient, because this approach not only reduced the polyphenols content in SCGH by about 22 %, but it also increased the PHAs yields by more than 25 %. Furthermore, this detoxification strategy also offers additional advantage, because extracted polyphenols might be used as valuable side-products of SCG conversion into PHAs. The polyphenols of SCG consist mainly of chlorogenic acids which provide many health benefits to humans [32], therefore polyphenols extracted from SCG may be utilized in manufacturing of functional foods or dietary supplements.

Thus PHAs can be produced using oil extracted from SCG as well as hydrolysate obtained by the hydrolysis of delipidised SCG. The conversion coefficient $Y_{PHA/SCG}$ for coffee oil utilization is

about 0.13 g/g, on the other hand, the utilization of hydrolysate by *B. cepacia* provides lower efficiency expressed by the $Y_{PHA/SCG}$ coefficient of 0.07 g/g. Therefore, by the combination of both approaches we can reach the conversion of SCG to PHAs of approx. 20 %. As was mentioned above, the solid residues after the oil extraction [30] but also after the hydrolysis (unpublished data) exhibit high calorific value – about 18 MJ/kg. It opens their possible use for the generation of heat and energy, which might be utilized to partially cover the energy demands of the up-stream fermentation as well as the down-stream process of PHAs production.

Conversion of SCG to yeast biomass enriched by carotenoids

Apart from PHAs, the hydrolysate of SCG can also be used as substrate for biotechnological production of alternative high value product – carotenoids. These pigments find numerous applications in food industry (food colorants, antioxidants, animal feed supplements) and also in cosmetics and pharmacy. Generally, the market demand for carotenoids is expected to increase substantially, since carotenoids exhibit significant anti-carcinogenic activities. Moreover, carotenoids play an important role in the prevention from various chronic diseases [33]. Aside from plants, carotenoids can also be produced by microorganisms such as yeasts, filamentous fungi, bacteria, algae and lichens. Similarly to PHAs, the biotechnological production of carotenoids can become industrially feasible if the costs of the process are minimized by the utilization of cheap carbon substrates such as waste products from agriculture or food industry [34].

We screened selected carotenogenic yeasts (*Rhodotorula glutinis*, *Cystofilobasidium capitatum*, *Rhodotorula mucilaginosa* and *Sporobolomyces roseus*) for their capability of SCGH utilization and carotenoids production. Among the yeasts strains tested, *S. roseus* revealed the highest biomass growth as well as pigments yields when utilizing the hydrolysate of delipidised SCG. Surprisingly, the yields were even higher than those from glucose based medium. Moreover, the profile of carotenoids produced by this strain contained the highest portion of β-carotene, which, due to its high vitamin A activity, is considered as one of the most valuable carotenoids [35].

Therefore, *S. roseus* was selected for further experiments in laboratory fermentor. The cultivations were performed in batch as well as fed-batch cultivation mode. In the fed-batch

cultivation, the pre-concentrated SCGH with sugars concentration of 370 g/l was used as a feeding solution. The pH-stat control was used as the substrate feeding strategy, due to reverse dependence of carbon source depletion and pH-value value of fermentation broth. Hence, a dose of feeding solution was introduced when the pH-value of media exceeded the value of 5.6. The fed-batch cultivation revealed significantly higher productivity and seemed to be more suitable for industrial production of carotenoids [35].

Conclusions

SCG – underutilised waste of coffee industry can be used as cheap substrate for the production of various products. We suggest utilizing this waste stream for the biotechnological production of PHAs and carotenoids – high value products with a number of applications. The concept of our technology (patent was submitted to Czech Patent Office) is provided in Fig. 2. The flow of the substrate throughout the technology can be controlled by market demands or other aspects. For instance, solid residues after oil extraction can be either used as fuel or converted to PHAs and carotenoids. Of course, the portfolio of the products of intended coffee-based bio-refinery can be much broader including those shown in Table 2. Nevertheless, we believe that coffee beverage will not be the only product of coffee industry in close future.

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Table 1 Composition of SCG

Parameter	Content [wt. %]
Cellulose	8.6 – 13.3
Hemicellulose	30 – 40
Proteins	6.7 -13.6
Oil	10 – 20
Lignin	25 - 33
Polyphenols	2.5
Caffeine	0.02

Data were combined from references 2 and 5.

Table 2. Suggested technologies for the valorisation of spent coffee grounds

Process/ Technology	**Product and its application**	**Reference**
Solid-liquid extraction by methanol	Phenolic substances for applications in health-care, food industry and pharmacy	9
Solid-liquid extraction by organic solvents	Phenolic substances for applications in health-care, food industry and pharmacy	10
Solid-liquid extraction by ethanol, combustion of solid residues	Phenolic substances and energy	6
Solid state fermentation by selected fungal strains	Phenolic substances for applications in health-care, food industry and pharmacy	11
Supercritical extraction of coffee oil	Application of extracted oil in cosmetic products	12
Oil extraction (organic solvents) and transesterification	Biodiesel	13
Oil extraction (organic solvents) and transesterification; fermentation	Biodiesel and ethanol	5
Oil extraction (organic solvents) and transesterification	Biodiesel	14
Microwave superheat water extraction	Galactomannans for applications in medicine and pharmacy	15
Hydrolysis by diluted acid and fermentation	Ethanol	16
Composting	Compost	17
Solid state fermentation	Edible mushroom *Flammulina velutipes*	18
Acid liquefaction	Polyols for production of polyurethanes	19
Solid state fermentation employing *Penicillium* sp.	Xylanase	20
Solid state fermentation employing *Neurospora crassa*	α-amylase	21
Sugar addition and fermentation	Alcoholic beverage	22
Modification of SCG with magnetic fluid	Magnetically modified spent coffee grounds for dyes removal	23

Table 3. Summary of PHAs production from spent coffee grounds

Microorganism	Substrate	Cultivation	CDW[1] (g/l)	PHA (g/l)	PHA (%)	PHA type	$Y_{P/S}$ (g/g)	Reference
Cupriavidus necator H16	Oil extracted from SCG	Batch, flasks	10.1 ± 0.2	7.0 ± 0.2	69.2 ± 1.5	PHB	0.35	27
Cupriavidus necator H16	Oil extracted from SCG	Batch, fermentor	29.4 ± 1.4	26.5 ± 1.6	90.1 ± 3.5	PHB	0.88	27
Cupriavidus necator H16	Oil extracted from SCG	Fed-batch, fermentor	55.4 ± 1.3	49.4 ± 2.1	89.1 ± 3.1	PHB	0.82	27
Burholderia cepacia	SCG hydrolysate	Batch, flasks	5.5 ± 0.4	3.1 ± 0.2	56.0 ± 7.8	P(HB-*co*-HV)[2]	0.24	29
Bacillus megaterium	SCG hydrolysate	Batch, flasks	3.4 ± 0.1	1.7 ± 0.1	51.1 ± 3.9	PHB	0.04	*u.p.*[3]

[1] CDW stands for cell dry weight

[2] Copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate

[3] *u.p.*- unpublished data

List of figure captions:

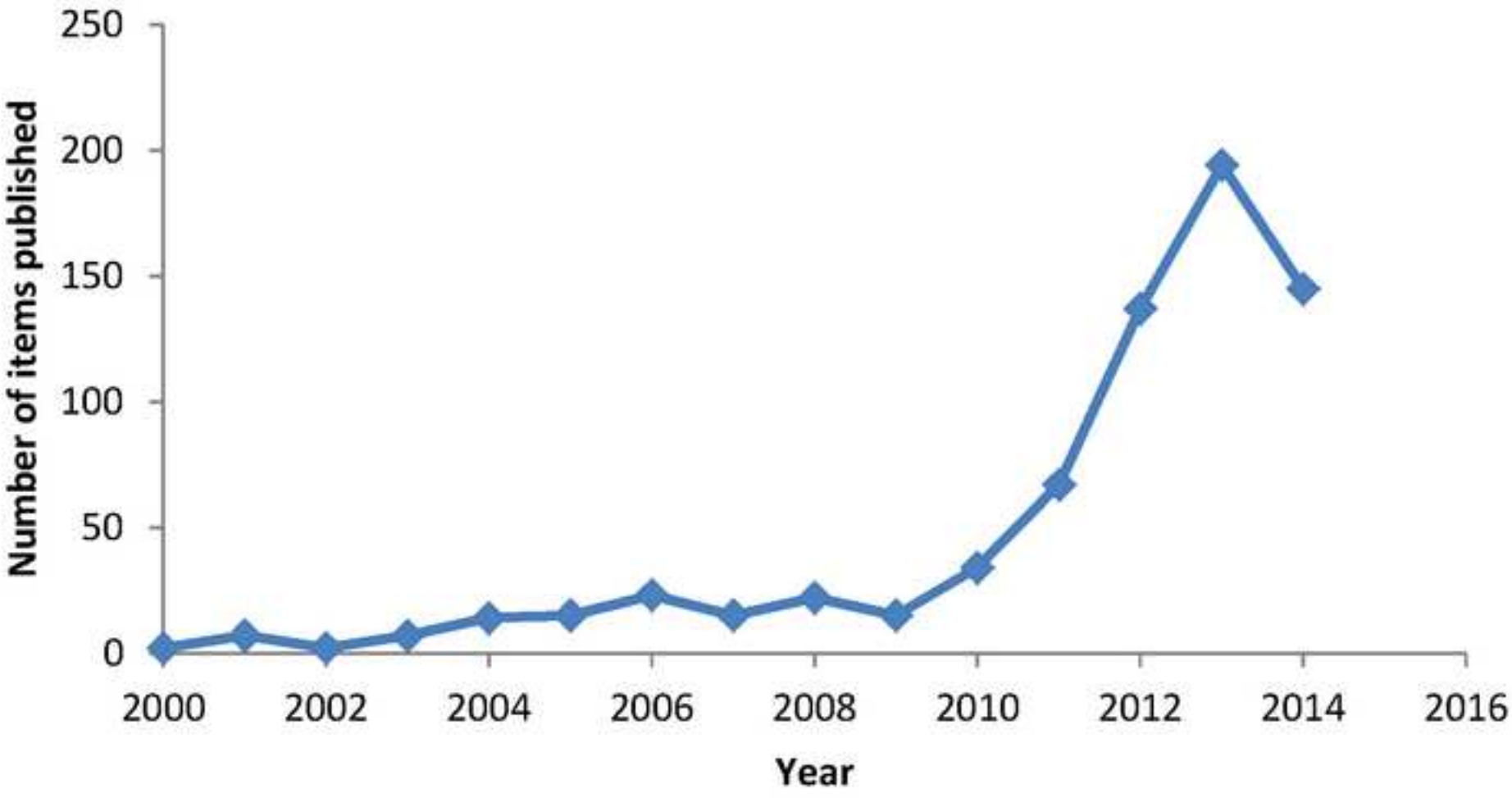
Fig. 1 Number of publications dealing with spent coffee grounds according to the Web of Knowledge (data from 27.7.2014)

Fig. 2 Diagram of potential process of SCG conversion into PHAs and carotenoids

Highlights

- Spent coffee grounds can be completely utilized using bio-refinery approach.
- Polyhydroxyalkanoates and carotenoids can be efficiently produced from spent coffee grounds employing selected microorganisms.
- Extraction of coffee polyphenols from spent coffee grounds improve efficiency biotechnological process and also provide additional high-value product.

Figure 1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2000
2002
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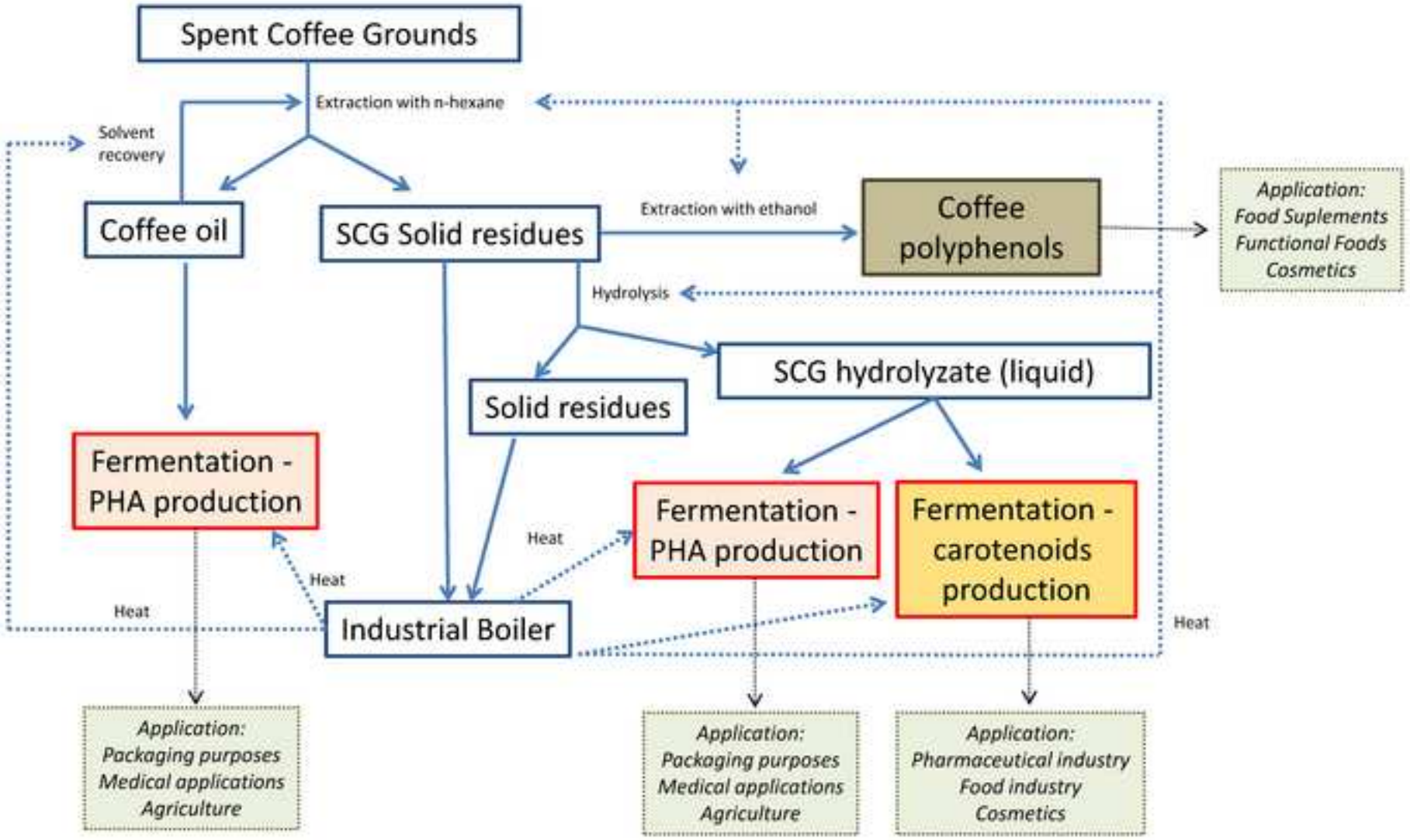
Year

Figure 2


Spent Coffee Grounds
Extraction with n-hexane
Solvent recovery
Coffee oil
SCG Solid residues
Extraction with ethanol
Coffee polyphenols
Application: Food Suplements Functional Foods Cosmetics
Hydrolysis
SCG hydrolyzate (liquid)
Solid residues
Fermentation - PHA production
Fermentation - PHA production
Fermentation - carotenoids production
Heat
Heat
Heat
Heat
Industrial Boiler
Application: Packaging purposes Medical applications Agriculture
Application: Packaging purposes Medical applications Agriculture
Application: Pharmaceutical industry Food industry Cosmetics