Reactive Separation of Vanillic Acid Using Tri-n-butyl Phosphate

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The aim of this study was to extract vanillic acid from an aqueous solution through reactive extraction. Vanillic acid is utilized in the food and beverage industry as a flavoring agent. Tri-n-butyl phosphate (TBP) served as the reactive extractant, while natural oils like sesame oil and groundnut oil acted as diluents. The recovery of vanillic acid from fermentation broth or wastewater poses challenges due to toxicity concerns. However, employing natural solvents in the extraction process can significantly reduce toxicity. Various parameters, such as extraction efficiency, loading ratio, distribution coefficient, and equilibrium complexation constant, were determined to evaluate the extent of extraction achieved. Groundnut oil emerged as more effective than sesame oil as a diluent, exhibiting a maximum distribution coefficient of 13.31 and an extraction efficiency of 93.01 % at 5.27 mmol L^{-1} of acid concentration and 40 % v/v of TBP. Additionally, the number of theoretical stages required for the reactive extraction system was calculated, resulting in five stages for groundnut oil and four stages for sesame oil.

Keywords
vanillic acid, distribution coefficient, reactive extraction, natural solvent, tri-n-butyl phosphate

Introduction

4-Hydroxy-3-methoxybenzoic acid, commonly known as vanillic acid, is a widely used flavoring agent known for its creamy and sweet fragrance.[1] It is usually found in crystal or powder form, appearing either yellow or white. Vanillic acid is a bioactive compound and a natural antioxidant, exhibiting good pharmacological activity. It is found in many foods, including grapes, cereal grains, and beans, as well as herbs such *Iodes cirrhosa* and *Angelica sinensis*.[2,3] Recent research suggests that vanillic acids could be used as bio-sourced monomers to synthesize novel polyesters. The resulting bio-sourced polyester showed similar mechanical and thermal properties to commercially produced polyethylene terephthalate.[4]

The vanillic acid market is projected to grow from USD 13687.95 in 2021 to USD 17060.21 in the next four years, with an estimated compound annual growth rate of 3.74 % from 2022 to 2027. The vanillic sector is primarily dominated by a few manufacturers. Quzhou Mingfeng, Donglian Nankai DlFlavor, and Solvay are the main producers of vanillic acids.[5]

Recent studies have shown that bioconversion, catalytic, and enzymatic processes can produce vanillic acid from veratric acid, vanillin, ferulic acid, and coniferyl alcohol.[1,6–9] Despite their potential, these studies face challenges such as low product concentrations, poor pH resistance impacting microbe health resulting in poor product quality, and slow fermentation rates. They also face difficulties in purifying the vanillic acids from wastewater streams and fermentation broth.[10,11]

Choosing the appropriate method for carboxylic acid recovery depends on its physio-chemical characteristics as each technique has its own drawbacks.[12].Volatile organic compounds, recognized as hazardous air pollutants due to their flammability and toxicity, present serious health, safety, and environmental risks, including issues related to process safety and waste management.[13]

Various methods, such as membrane technology,[14] liquid-liquid extraction,[15] chromatographic separation[16] and adsorption[17] can be employed to extract phenolic compounds from aqueous media. Examples of membrane-based technology include nanofiltration, osmotic distillation, microfiltration, and vacuum membrane distillation, all used in the removal of phenolic compounds from aqueous streams.[14]

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The reactive extraction technique has proven successful in recovering carboxylic acid from diluted fermentation broths or aqueous streams.[18,19] This technique separates components based on their relative solubilities and reversible reactions with the extractant in two immiscible fluids. The reversible nature of reactive extraction allows it to be more efficient and effective than traditional liquid-liquid methods.[20] Recent research has utilized reactive extraction in the separation of organic acids from fermentation broth/waste, benefiting from the extractant's easy recovery and recyclability.[21–23] As an effective separation method, reactive extraction is valued in several applications for its low-cost and straightforward procedure. However, the main challenge in using reactive extraction for acid recovery lies in finding an effective and affordable extractant and diluent. Reactive extraction utilizes composite solvents containing an extractant that forms a complex with the acid, and a diluent to control physical properties, and to, specifically, facilitate complexation. Tri-n-butylphosphate (TBP) was chosen as the extractant to enhance acid solvation in the diluent.[15] While natural oils may not exhibit optimal performance, their low toxicity, cost-effectiveness, biodegradability, and easy availability make them a viable choice for separating vanillic acid from the aqueous phase. The separated vanillic acid can also be utilized for food and pharmaceutical purposes without concerns about contamination from toxic solvents.

Several studies have explored the use of natural oils as diluents for the recovery of carboxylic acids, such as lactic acid,[24,25] glutaric acid,[26] caproic acid,[27,28] picolinic acid,[29,30] nicotinic acid,[31,32] itaconic acid,[33] propionic acid,[34,35] protocatechuic acid,[36,37] levulinic acid,[23] gallic acid,[38,39] acrylic acid,[40,41] malonic acid[42] and vanillic acid.[43,44]

In earlier studies, Dandekar *et al.* utilized tri-n-butyl phosphate–soybean oil as the extractant-diluent combination for the recovery of vanillic acid by reactive extraction.[43] The maximum extraction efficiency was reported to be 74.91 % for soybean oil. Vanillic acid extraction efficiency exceeded 80 % when using aqueous two-phase systems with ionic liquids.[45] Wang *et al.* demonstrated nearly 84 % efficiency in separating vanillic acid using 1-hexyl-3-methylimidazolium hexafluorophosphate, a hydrophobic ionic liquid.[3] In chemical equilibrium studies, tri-n butyl phosphate was combined with groundnut oil and sesame oil to recover vanillic acids at concentrations between 1.09 – 5.27 mmol L^{-1}. This research aims to investigate the vanillic acid extraction process, and make it more affordable and less toxic by employing bio-based natural oils, which are cheaper than conventional solvents. To describe the experimental results, the following parameters were estimated: equilibrium complexation constants (K_E), distribution coefficients (K_D), loading ratio (z), and extraction efficiency (η %). The experiments were conducted at atmospheric pressure (1 atm), and a temperature of 298.15 K.

Materials and methods

Chemicals

Oxygen-bearing phosphorous-bonded TBP ($C_{12}H_2O_4P$) with a mass fraction of 0.99 was procured from Spectrochem India. TBP has a molecular mass of 266 g mol^{-1} and a density of 975 kg m^{-3}. Vanillic acid of laboratory grade was obtained from SRL India Pvt. Ltd. Mumbai, India, and used to prepare vanillic acids in an aqueous phase at different concentrations. Natural diluents were sourced from the local market, and SD Fine Chem Ltd., India, provided laboratory-grade sodium hydroxide for colorimetric titration. Oxalic acids with a mass fraction of 0.99 were used for the standardization of NaOH with phenolphthalein.

Phenolphthalein, the colorimetric indicator for titration, was supplied by Merck Life Science Pvt. Ltd. Mumbai (India).

Experimental method

In demineralized water, a specific amount of vanillic acid powder was dissolved in an aqueous solution with varying concentrations ranging from 1.09 to 5.27 mmol L^{-1}. The concentration range of the acid was deliberately chosen to replicate the conditions found in the fermentation broth, where vanillic acid is produced from ferulic acid through biotransformation using *Halomonas elangata.*[7] To conduct the experiment, conical flasks were utilized and placed in an orbital shaking incubator. The incubator contained an equal volume of TBP (tri-n-butyl phosphate, 365 – 1460 mmol L^{-1}) mixed with diluents for the organic phase, and freshly prepared vanillic acid for the aqueous phase, both in 10 mL solutions. The specific incubator model used was the S-24BL manufactured by REMI India. The incubator was set to operate continuously at 150 rpm, at a temperature of 298.15 K, and under atmospheric pressure for a duration of five hours. Once the desired separation between the two phases was achieved, the samples were transferred to Falcon tubes. Subsequently, they were centrifuged at 5000 rpm for 600 seconds to further separate the two phases. After carefully isolating the water phase, the concentration of vanillic acid in the aqueous phase was determined by titrating it with a solution of 0.5 mmol L^{-1} NaOH. Applying a mass balance calcula-

tion determined the concentration of vanillic acid in the organic phase.

Statistical and uncertainty analysis

To conduct the statistical uncertainty analysis, certain experiments had to be run two or three times. The results consistently showed an error rate below 2 %, a deviation of less than 1 %, and a confidence interval of 98 %. The following equation was used to attain experimental uncertainty within $x \pm 0.001$.

$$\mu(x) = \sqrt{\frac{\sum_{i=\mathrm{N}}^{\mathrm{N}} \left(x_i - \bar{x}\right)^2}{(\mathrm{N}-1)}} \tag{1}$$

where N is the number of experiments performed, $\bar{x}$ is the mean values of the experimental readings, and x_i is the values of the experimental readings.

Results and discussion

The initial phase of our study involved a physical extraction equilibrium study of vanillic acid using natural oils, specifically, sesame oil and groundnut oil, as the organic phase. In physical extraction, the molecules of vanillic acid distribute between the organic and aqueous phase without forming any chemical complexes between vanillic acid and the organic phase.[46] The distribution ratio (for an equal volume of phases) is equal to the ratio of the equilibrium concentration of vanillic acid in the organic phase $[\mathrm{VA}]_{\mathrm{org}}$, to its equilibrium concentration in the aqueous phase $[\mathrm{VA}]_{\mathrm{aq}}$, denoted as K_D.[46] Equations 2 and 3 were employed to calculate the distribution coefficient and extraction efficiency, providing insight into the performance of the physical extraction of vanillic acid.

$$K_D = \frac{[\mathrm{VA}]_{\mathrm{org}}}{[\mathrm{VA}]_{\mathrm{aq}}} \tag{2}$$

$$\eta\% = \frac{K_D}{1 + K_D} \cdot 100 \tag{3}$$

Sesame oil exhibited the highest distribution coefficient of 1.38 and extraction efficiency of 57.99 % at a concentration of 1 mmol L^{-1}, which can be attributed to the presence of sesamol. Sesamol contains an active hydroxy group that allows for hydrogen bonding with the solute, resulting in better extraction performance ranging from 46.99 % to 57.99 %. Sesame oil is composed of roughly 40 % oleic acid (mono-unsaturated fatty acids) and 43 % linolenic acid (poly-unsaturated fatty acid) as opposed to the composition of groundnut oil being 49 % oleic acid and 31 % linolenic acid.[46,48]

With an increase in the initial concentration of vanillic acid, sesame oil experiences a decrease in both the distribution coefficient and extraction efficiency. This could be attributed to sesame oil's limited capacity to accommodate the increasing number of acid molecules in the organic phase. This limitation arises from the fixed number of sites available for hydrogen bonding with the solute molecules.

Similarly, groundnut oil demonstrated a decreasing trend in extraction efficiency and distribution coefficient with increasing initial acid concentration. At an initial acid concentration of 1 mmol L^{-1}, groundnut oil achieved the highest separation parameters with an extraction efficiency of 43.61 % and a distribution coefficient of 0.77 at 1 mmol L^{-1}.

Although groundnut oil contains a small percentage of polyunsaturated fatty acids (specifically, linoleic acid) compared to sesame oil, groundnut oil exhibited a lower average distribution ratio value of 0.702 and an average separation efficiency value of 41.27 % as compared to sesame oils average extraction value and distribution ratio value of 52.66 % and 1.112.[46]

The results of the physical extraction study revealed poor separation parameter values, indicating the necessity for an extractant to enhance the extraction of vanillic acid from the aqueous phase. Tri-n-butyl phosphate was chosen as the extractant, with volumetric concentrations varying from 10 % to 40 % (365–1460 mmol L^{-1}). TBP, compared to other widely used extractants like trioctylamine and Aliquat 336, is less complicated to handle due to its lower viscosity and better flow properties. Its interaction with diluents boosts the effectiveness of extraction. When utilizing organophosphorus compounds as extractants to increase extraction efficiency, thermodynamic stability is also crucial.[47] TBP's specific interactions with electron-acceptor and electron-donor groups include complex creation and self-association of vanillic acid molecules with diluent molecules. Therefore, it can be utilized to increase the accuracy of the extraction procedure. Vanillic acid, with a pK_a value of 4.16, shows that it is a stronger acid compared to other carboxylic acids; it also has a low solubility in water of 5.7 g L^{-1}.[50] These properties of vanillic acid suggest that it is easier to separate, potentially resulting in favorable separation parameter values.

Table 1 provides the data on the composition of the natural oils used as the diluents in this study. Groundnut oil has a lower percentage of polyunsaturated fatty acids, such as linoleic and linolenic acid, compared to sesame oil. However, it has a higher amount of monounsaturated fatty acids (oleic acid) and long-chain saturated fatty acids like archadic acid, behenic acid, and lignoceric acid.

Table 1 – *Composition of the natural oils used in this study*

Natural oil	Monounsaturated fatty acids (%)	Polyunsaturated fatty acids (%)		Long-chain saturated fatty acids (%)		
	Oleic acid	Linoleic	Linolenic	Archadic	Behenic	Lignoceric
Groundnut oil	49.63	31.52	0.64	1.07	2.86	1.3
Sesame oil	40.18	43.46	0.56	0.57	0.08	0

Table 2 – *Reactive extraction of vanillic acid (5.27 mmol L^{-1}) using higher concentrations of TBP in groundnut oil*

TBP Vol %	$[VA]_{aq}$, (mmol L^{-1})	$[VA]_{org}$, (mmol L^{-1})	η (%)	K_D
20	0.693	4.576	86.85	6.61
40	0.368	4.900	93.01	13.31
60	0.442	4.827	91.61	10.92
80	0.519	4.750	90.14	9.15
100	0.637	4.632	87.90	7.26

For this study, five different initial acid concentrations ranging from 1.09 to 5.27 mmol L^{-1} were considered. The experimental findings of reactive extraction using groundnut oil with higher TBP concentrations ranging from 365 to 3650 mmol L^{-1} are presented in Table 2. The extraction efficiency decreased at higher concentrations of extractant, suggesting insufficient diluent molecules available to dissolve the TBP-acid molecules, resulting in reduced separation performance. A diluent's role is to improve the physicochemical properties of the solvent system, such that it facilitates the dissolution of the TBP-acid complex, thus enhancing the extraction process.[28,51]

When TBP served as the entire organic phase, the extraction efficiency was 88 %, possibly due to the absence of a diluent which could have solvated the TBP-acid complex much more easily, leading to higher separation parameter values. This suggests that the separation process is enhanced by the introduction of a diluent.

The values of various separation parameters are presented in Tables 3 and 4 for vanillic acid reactive extraction using TBP. The isotherms for reactive extraction of vanillic acid with TBP and varying amounts of vanillic acids are depicted in Figs. 1 and 2. When groundnut oil and sesame oil were mixed with TBP, the equilibrium concentrations for vanillic acid in the aqueous and organic phases showed linear variations. TBP led to an increase in distribution coefficient (K_D) and extraction efficiency (η %) for both natural diluents.

The effects of various separation parameters were initially investigated for groundnut oil. At 40 % v/v, the maximum η % was observed for groundnut oil; hence, this concentration of TBP was chosen as the threshold, and the extraction performance of groundnut oil was compared to that of sesame oil in this study.

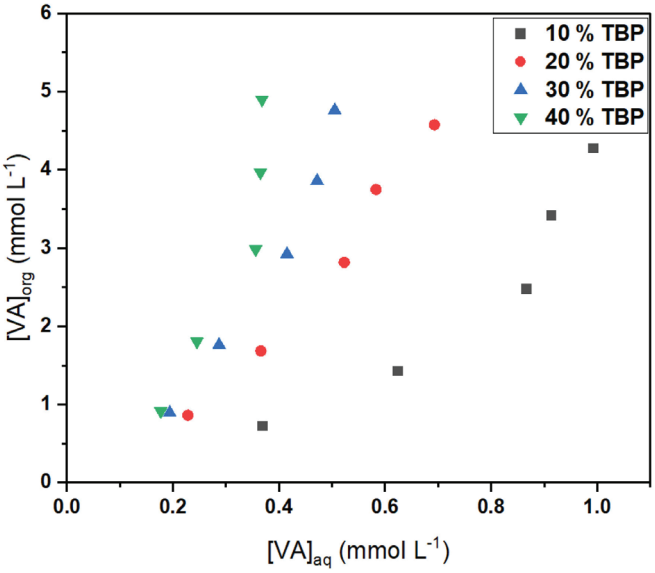

Fig. 1 – *Reactive extraction equilibria of vanillic acid using TBP in groundnut oil*

In all solvent-TBP systems, as the initial concentration of vanillic acid increased from 1.09 mmol L^{-1} to 5.27 mmol L^{-1} over a fixed TBP concentration, the distribution ratio (K_D) and extraction efficiency also increased. The relationship between the initial vanillic acid concentration and the distribution ratio (K_D) for the diluents used at different TBP concentrations is depicted in Figs. 3 and 4. Higher distribution coefficients, caused by lower solvent flowrates, translate into lower recycling rates, as well as purification and separation costs.

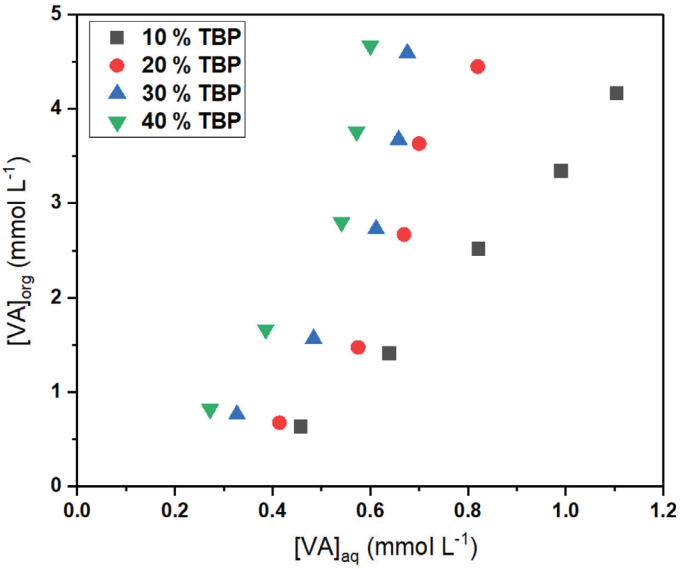

Fig. 2 – *Reactive extraction equilibria of vanillic acid using TBP in sesame oil*

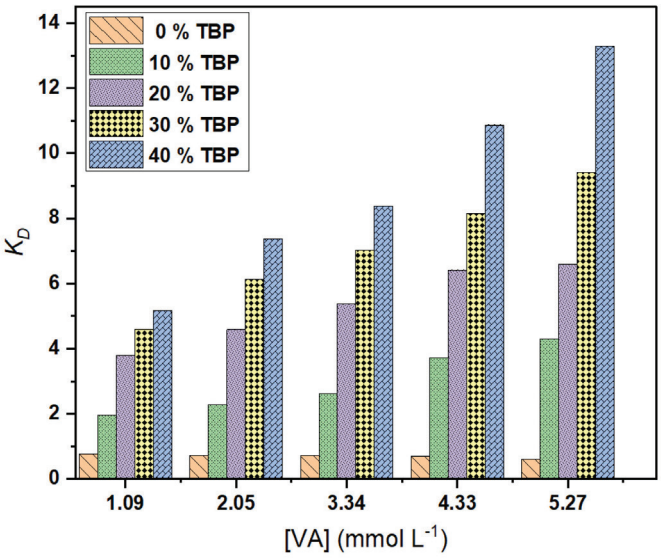

Fig. 3 – *Effect of initial acid concentration on the distribution ratio for TBP in groundnut oil at 298.15 K*

Tables 3 and 4 demonstrate that K_D values significantly increased as TBP concentrations increased in different diluents. When the extractant quantity increased from 10 % to 40 % v/v, extractant efficiency and distribution ratios improved. When using groundnut oil, the maximum extraction efficiency of 93.01 percent was observed at a TBP concentration of 1460 mmol L^{-1}. It could be concluded that the distribution ratio for the recovery of vanillic acid from the aqueous stream and the ex-

Table 3 – *Reactive extraction of vanillic acid using TBP in groundnut oil*

TBP Vol %	[VA] (mmol L^{-1})	$[VA]_{aq}$, (mmol L^{-1})	$[VA]_{org}$, (mmol L^{-1})	η (%)	K_D	$z \cdot 10^{-3}$	$K_E \cdot 10^{-2}$ (L $mmol^{-1}$)
10	1.09	0.369	0.722	66.19	1.95	2.0	1.458
	2.05	0.624	1.426	69.54	2.28	3.9	
	3.34	0.867	2.473	72.39	2.62	6.2	
	4.33	0.914	3.419	78.89	3.73	9.4	
	5.27	0.993	4.276	81.15	4.31	11.7	
20	1.09	0.228	0.863	79.10	3.79	1.2	1.120
	2.05	0.366	1.684	82.15	4.60	2.3	
	3.34	0.523	2.817	84.35	5.39	3.9	
	4.33	0.583	3.750	86.53	6.43	5.1	
	5.27	0.693	4.576	86.85	6.61	6.3	
30	1.09	0.194	0.897	82.18	4.61	0.8	1.071
	2.05	0.287	1.763	85.99	6.14	1.6	
	3.34	0.415	2.925	87.56	7.04	2.7	
	4.33	0.472	3.860	89.09	8.17	3.5	
	5.27	0.505	4.763	90.41	9.43	4.3	
40	1.09	0.177	0.914	83.79	5.17	0.6	1.139
	2.05	0.245	1.805	88.06	7.38	1.2	
	3.34	0.356	2.984	89.34	8.39	2.0	
	4.33	0.365	3.968	91.58	10.88	2.7	
	5.27	0.368	4.900	93.01	13.31	3.4	

Table 4 – *Reactive extraction of vanillic acid using TBP in sesame oil*

TBP Vol %	[VA] (mmol L^{-1})	$[VA]_{aq}$, (mmol L^{-1})	$[VA]_{org}$, (mmol L^{-1})	η (%)	K_D	$z \cdot 10^{-3}$	$K_E \cdot 10^{-2}$ (L $mmol^{-1}$)
10	1.09	0.418	0.673	61.70	1.61	1.7	1.43
	2.05	0.639	1.411	68.81	2.21	3.9	
	3.34	0.822	2.518	75.40	3.07	6.9	
	4.33	0.991	3.342	77.14	3.37	9.2	
	5.27	1.105	4.164	79.04	3.77	11.4	
20	1.09	0.414	0.677	62.03	1.63	0.9	1.35
	2.05	0.575	1.475	71.96	2.57	2.0	
	3.34	0.669	2.671	79.98	3.99	3.7	
	4.33	0.700	3.633	83.85	5.19	5.0	
	5.27	0.820	4.449	84.45	5.43	6.1	
30	1.09	0.327	0.764	70.03	2.34	0.7	0.913
	2.05	0.484	1.566	76.41	3.24	1.4	
	3.34	0.612	2.728	81.66	4.45	2.5	
	4.33	0.658	3.675	84.81	5.58	3.4	
	5.27	0.676	4.593	87.17	6.80	4.2	
40	1.09	0.272	0.819	75.11	3.02	0.6	0.725
	2.05	0.386	1.664	81.18	4.31	1.1	
	3.34	0.541	2.799	83.81	5.18	1.9	
	4.33	0.572	3.760	86.79	6.57	2.6	
	5.27	0.600	4.669	88.62	7.79	3.2	

traction performance are directly influenced by the concentrations of TBP. Long-chain saturated fatty acids have greater molecular weight compared to other classes of fatty acids, and their presence in oils indicates longer chain lengths, which are hydrogen-dense. This explains why groundnut oil has a higher molecular weight and viscosity compared to sesame oil. This configuration of fatty acids makes groundnut oil more capable of housing the vanillic acid-TBP complexes. This could be the possible reason why groundnut oil has shown much better recovery of vanillic acid during reactive extraction compared to sesame oil. However, this phenomenon was found to be reverse during physical extraction, explaining that groundnut oil can solvate the acid-extractant complex more easily than just the acid molecules.

During chemical extraction, tri-n-butyl phosphate ([E]) and vanillic acid ([VA]) undergo a complexation process that results in a 1:1 ([E:VA]) complex, as seen in the following reaction:

$$n[VA]_{aq} + m[E]_{org} \xrightleftharpoons{K_{E(1:1)}} [(E)_m:(VA)_n]_{org}$$

Equilibrium complexation constant for the reaction can be described as:

$$K_{E(1:1)} = \frac{[(E)_m : (VA)_n]_{org}}{[E]^m_{org}[VA]^n_{org}} \quad (4)$$

The creation of complexes involving vanillic acid and the extractant (TBP) is described by the equilibrium complexation constant (K_E).

Vanillic acid and extractant quantities in the organic phase are compared by their loading ratio (z). This ratio, which can be expressed as follows in Equation 5, demonstrates how much acid is transferred from the aqueous phase into the organic phase.

$$z = \frac{[VA]_{org}}{[E]_{1:1org}} \quad (5)$$

Equation 4 was employed to calculate the loading ratios for each of the extractant-diluent systems shown in Tables 3 and 4. The main factors affecting the loading ratio are the aqueous acid concentration and its extractability, or the strength of the acid-base interaction. Additionally, the stoichiometry of the overall reaction in the organic phase is determined

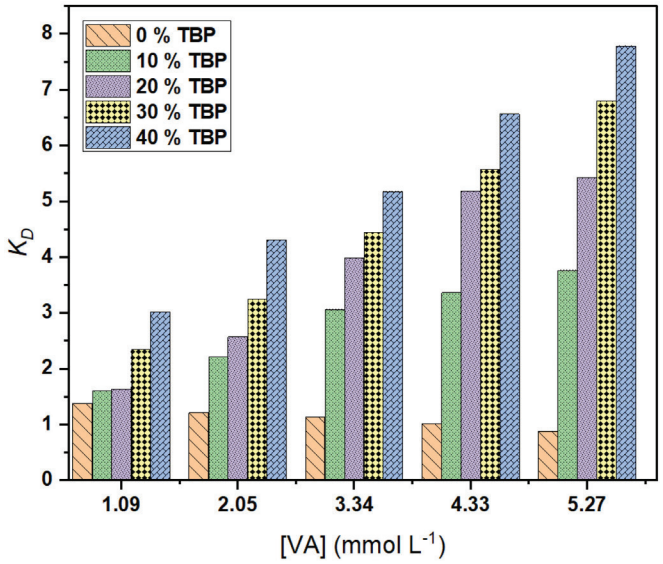

Fig. 4 – *Effect of initial acid concentration on the distribution ratio for TBP in sesame oil at 298.15 K*

by the loading ratio. Depending on the loading ratio (*z*), the acid:extractant complex may form in the organic phase in either a 1:1 or 2:1 ratio. Since the values of *z* in the current investigation are below 0.5, only 1:1 acid:extractant complex formation occurs, and overloading does not occur.[52] Lower extractant amounts and stronger initial acid concentrations were also associated with higher loading ratios. With a fixed extractant concentration, it can be demonstrated that the loading ratio increased with an increment in acid concentration, and vice versa with an increase in extractant concentration for a fixed acid concentration. For various extractant-diluent combinations, the maximum loading ratio number was determined to be z = 0.0117 for groundnut oil and z = 0.0114 for sesame oil at 10 % v/v TBP concentration and an initial acid concentration of 5.27 mmol L^{-1}.

For the current investigation, Equation 6 was applied to estimate the K_E values for various diluents:

$$\frac{z}{1-z} = K_E[\mathrm{VA}]_{\mathrm{aq}} \tag{6}$$

Tables 3 and 4 provide the values of K_E for the various diluents taken into account in the current study. The values of K_E are determined by the slope of a linear graph between $z/(1–z)$ and $[\mathrm{VA}]_{\mathrm{aq}}$. The K_E values were found to range from 0.0107 to 0.0145 L mmol^{-1} for groundnut oil, and 0.0725 to 0.0143 L mmol^{-1} for sesame oil for the various extractant concentrations (365–1460 mmol L^{-1}). The higher the K_E value, the better the stability of the complex formed, indicating that groundnut, as the diluent, provides a better medium for which TBP-acid complex can be solvated, compared to sesame oil.

Furthermore, in the context of in-situ fermentation separation, it is important to consider the potential toxicity of TBP. TBP can have adverse effects on microbial growth and metabolism. Microbes involved in fermentation processes are particularly sensitive to the presence of toxic compounds, as they can impact growth rates, product yields, and overall fermentation performance. Therefore, when exploring the use of vanillic acid extracted using TBP in fermentation applications, it is imperative to assess and address the potential toxicity of residual TBP to the fermentation microbes. Conducting microbial growth studies and toxicity assays can provide insights into the tolerance of the chosen fermentation organisms to TBP, and guide decisions on the suitability of the extracted compound for fermentation processes.

Reactive extraction offers advantages in separating compounds from fermentation broth, but poses challenges due to impurities like proteins and amino acids. These impurities can lead to complex interactions with the extractant, affecting selectivity and yield. Phase separation issues and slower kinetics can arise, potentially causing contaminant carryover and reduced selectivity. Strategies to address these challenges include pretreatment of the broth, optimizing extractant and conditions, incorporating purification steps, and rigorous characterization and analysis techniques.

The practicality of vanillic acid extraction using TBP in various diluents can be assessed using the $(\mathrm{S/F})_{\mathrm{min}}$ (minimum solvent-to-feed flow) ratio, which subsequently determines how many stages are absolutely essential for counter-current extraction. Equation 7 was used to determine the $(\mathrm{S/F})_{\mathrm{min}}$ ratio.

$$\left(\frac{\mathrm{S}}{\mathrm{F}}\right)_{\mathrm{min}} = \frac{x_{in} - x_{out}}{K_D x_{in} - y_{in}} \tag{7}$$

The variables x_{in} and x_{out} in Equation (7) represent the concentrations of vanillic acid in the feed and raffinate phases, respectively. On the other hand, the initial concentration of vanillic acid in the extract phase is denoted by y_{in}. The value of $(\mathrm{S/F})_{\mathrm{actual}}$ corresponds to 1.5 times the minimum solvent-to-feed ratio required for an extraction process with a fixed number of extraction stages.[37]

The modified Kremser equation can be used to calculate the number of theoretical stages (NTS) in the procedure for extraction by counter-current configuration.[37] It can be stated as:

$$\mathrm{NTS} = \frac{\ln\left(\left(\frac{x_{in} - y_{in}/K_D}{x_{out} - y_{in}/K_D}\right)\left(1 - 1/E_x\right) + \frac{1}{E_x}\right)}{\ln\left(E_x\right)} \tag{8}$$

Table 5 – *Minimum solvent-to-feed (S/F) ratio and number of theoretical stages (NTS) for the recovery of vanillic acid*

Diluent	x_{in} (mmol L^{-1})	x_{out} (mmol L^{-1})	K_D	$(S/F)_{min}$	$(S/F)_{actual}$	E_x	NTS
Groundnut oil	1.09	0.17	5.17	0.162	0.243	1.256	3.15
	2.05	0.24	7.38	0.119	0.178	1.321	3.69
	3.34	0.35	8.38	0.106	0.159	1.340	3.89
	4.33	0.36	10.87	0.084	0.126	1.373	4.33
	5.27	0.36	13.31	0.069	0.104	1.395	4.69
Sesame oil	1.09	0.27	3.02	0.248	0.373	1.126	2.44
	2.05	0.38	4.31	0.188	0.282	1.217	2.90
	3.34	0.54	5.17	0.161	0.242	1.257	3.15
	4.33	0.57	6.56	0.132	0.198	1.301	3.50
	5.27	0.59	7.78	0.113	0.170	1.329	3.77

where Equation 9 defines the extraction factor E_x

$$E_x = K_D\left(\frac{S}{F}\right)_{actual} \quad (9)$$

For this study, the parameters were evaluated using a concentration of 1460 mmol L^{-1} of tri-n-butyl phosphate (TBP) in vanillic acid, considering various diluents because the highest extraction efficiency was obtained at that specific point. These parameters were utilized to determine the minimum S/F (solvent-to-feed) ratio and the NTS (number of theoretical stages), which directly impact the highest possible distribution and recovery of vanillic acid. It would take five theoretical stages to extract vanillic acid using groundnut oil and four theoretical stages if sesame oil is used, with the predicted efficiency in a continuous extraction column, according to the data in Tables 3 and 4. Table 5 lists the S/F and NTS values for each extractant-diluent system.

Conclusion

At 298 K, equilibrium investigations of reactive separation of vanillic acid with TBP as the extractant and natural oils such as groundnut oil and sesame oil as the diluents were performed. The added advantage of using natural oils as diluents is that they are less toxic, more sustainable and environmentally friendly, but also showed separation results which were on par with the conventional solvents. Calculations were made for parameters including loading ratio, equilibrium complexation constants, distribution coefficient, and extraction efficiency. The results obtained from reactive extraction were much better than the physical extraction, as expected. Sesame oil had shown better separation performance than groundnut oil for physical extraction. However, to the opposite was observed in reactive extraction where groundnut oil was found to be a better diluent than sesame oil with greater extraction efficiency and distribution ratio (93.01 %, 13.31) > (88.62 %, 7.79) for 40 % v/v of TBP, meaning that groundnut oil is more capable than sesame oil to solvate the acid-extractant complex. The loading ratios were found to be less than 0.5 for both the extractant-diluent combinations, suggesting that there was no overloading of acid-TBP complexes, and the complex formed was in the ratio of 1:1. The equilibrium complexation constants (K_E) were in the range 0.0107–0.0145 L mmol^{-1} for groundnut oil, and 0.0072–0.0143 L mmol^{-1} for sesame oil. Groundnut oil was found to have better capability of solvating the acid-extractant complex than sesame oil; therefore, groundnut oil is a better option as a diluent than the latter. The number of theoretical stages (NTS) required and the minimum solvent-to-feed ratio was computed, as these data can be useful for designing a continuous extraction column.

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