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Valorization of Natural Tomato Wastes in the Reduction of Prochiral Ketones

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ABSTRACT

In recent years, a great amount of attention has been paid to asymmetric synthesis of chiral synthons, the demand for which is increasing as precursors in the development of modern drugs and agrochemicals. Chiral alcohols are one of the many well-known synthons and can be obtained from the corresponding prochiral ketones by asymmetric reduction.. In this work,Organic chemical bioconversion of prochiral ketones to chiral alcohols with natural tomatoes food waste was made in water and glycerol and compared to known strains of waste and microorganisms (*e.g. Baker's Yeast , Daucus carota*). These enzymatic reduction attempts lead to best results with excellent enantiomeric excess (ee) in *S* configuration.

Keywords: Tomato wastes, enzymatic reduction, secondary alcohols, enantioselectivity.

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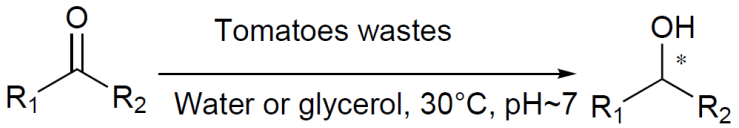
INTRODUCTION

The production and the preservation of the environment without the alteration of the soil and water space during the production or manufacture should be the main condition and one of the greatest preoccupation of public administration.[1] In the East of Algeria, many tomato processing factories are installed around Seybouse Valley and produce a lot of food wastes, source of soil and water pollution of this fertile agricultural plain. In addition to their use as a fertilizer of natural composts, biogas ... etc.[2–4] these wastes can be recycled as valuable organic products with many features of bioconversion in organic and green chemistry and other uses. [5, 6]. It is well know that vegetable and fruit processing by-products, wastes and effluents typically consist of high amounts of proteins, sugars and lipids[7, 8] can be sources of enzyme activity and can be used as a biocatalyst product in various organic chemistry reactions.[9, 10]

On the other hand, chemical synthesis of organic molecules with chiral centers often gives a racemic mixture that is difficult and expensive to separate, presenting a major obstacle to accede to many fine active biologically chemical products. Among the many types of biocatalysts used, the carbonyl reductases were used for preparing optically active alcohols from various prochiral ketones [11–14] For example, in organic synthesis, the reduction of a prochiral ketone to the secondary alcohol, [15–17] is an enantioselective manner among others that could be explored in this valorization. [18, 19].



R_1= Phenyl, 4-Methylphenyl, 4-Nitrophenyl, 4-Hydroxyphenyl. R_2= Methyl.
R_1= Phenyl. R_2= 3-chloroethyl.
R_1= Pentyl, Methyl. R_2= Ethyl.

Figure 1 Prochiral ketone reduction

In particular, when the enantioselectivity of the alcohol produced is very high, [20–22] and has a great interest as building block [23, 24] for accede to potentially biologically active products. [25, 26]

MATERIELS AND METHODS

Tomato wastes are freshly collected in plant manufacture [59] and are directly used without any processing in same day. The reduction's attempts with tomato fruit [60] bought to local market and used after having been cut into small slices are best and comparable with tomato wastes. The conversion and products ratio is determined by GLC.

The enantiomeric composition of product was established by chiral column FS Cyclodex beta- I/P over Shimatzu GC 4010 and P1000-LED polarimeter with precision of lecture 0.05◦. Configuration of product was determined by the sign of the specific rotation and in accordance with literature. [61, 62]

EXPERIMENTAL

A general procedure used for Bioreduction with

Tomato wastes are in accordance with reference cited [53]. The results are detailed in the table. Tomatoes wastes (5 g), or a tomato cut into small dices (50g) was placed in Erlenmeyer flasks with 50 mL of solvent (water or glycerol)[54]. (50 mg) of the substrate [55] was added to the suspension of the tomato, and the resulting mixture was agitated by a mechanical stirrer with the agitation speed at 300 rpm. The temperature of the reaction system was kept at 30 ◦C. The process of the reaction was monitored by GC and comparison with product reduces with chemical method. [56, 57, 58]

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After completion of the reaction, the suspension was filtered off and the filtrate was extracted with ethyl acetate ((3x40 mL for water and 3x20 mL for glycerol); then the combined organic layer was dried over anhydrous MgSO4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (dichloromethane: methanol = 9:1).

RESULTS AND DISCUSSION

The aim of this work was to make several attempts with raw food waste on various prochiral ketones, to know the reduction capacity of waste, optimize the reaction conditions and the solvent use.[27–29] Our interest for tomato waste is dictated by the lack of serious and comprehensive studies of this plant in the field of enzyme activity such as reductase.[30] For these BioReagents / catalysts widely applied in synthetic chemistry, they need to operate in retaining their selectivity in more compatible with organic compounds and solvents. For example: the Baker's yeast is known to perform a variety of transformations in synthetic organic chemistry, such as reduction of carbonyl groups.

However, its use in this field has been limited by the necessity of employing aqueous solvent systems. They exist several works related to these problems. [31, 32] The main advantage of using organic solvents is the ease with which the product can be extracted. Alternative biocatalyst research with the same activity among domestic waste, could be an interesting approach. In this paper, we are particularly interested in the tomato activity (Lycopersicum esculentum). So, we studied the tomato reduction activity in both solvents (water and glycerol). The choice of substrate is made by disposability and the structure of prochiral linear ketone : octan-3-one (III) and butan-2-one (V), aromatic cycle: acetophenone (I), aromatic cycle with heteroatome: p-nitroacetophenone (IV) 3-chloro-1-phenylpropan-1- one (VI), and aromatic cycle with substituted alkyl: 4-methylacetophenone (II). The Table 1 below shows some attempts of reduction with Tomato (T) over seven prochiral ketones cited before. The global results of these attempts, shows that enantiomeric excesses in water or glycerol as solvent are the best and (S) conversion is according with Prelog's rule. results.

N°	Red.	Sub.	Conv.% t(h)		ee (%)		Conf.	
			Wat.	Gly.	Wat.	Gly.	Wat.	Gly.
1	T	I	40(96)	89(96)	>99	>99	S	S
2	T.	II	40(96)	87(96)	>99	>99	S	S
3	T.	III	<2(180)	12(96)	-	>99	-	S
4	T.	IV	36(96)	29(96)	>99	>99	S	S
5	T.	V	<2(180)	<3(96)	-	-	-	-
6	T.	VI	100(72)	<1(120)	>99	>99	S	S

Table 1 The reduction tests with Tomatoes wastes on prochiral ketones in water and glycerol.

Acetophenone (I), 4-methylacetophenone ((II)),Octan-3-one (III), p-nitroacetophenone (IV), butan-2-one (V), 3-chloro-1-phenylpropan-1-one (VI).

Reactivity

All the essays realised for understanding various aspects of the reactivity in the enzymatic pocket [33, 34], according to the nature of the substituents R1 and R2 of the substrate (see Fig.1), and explain if either the volume or electronic character or the both can influence reactivity and selectivity. So, if the selectivity does not alter in any case, the reaction kinetic were affected by structure and nature of the substrate; we think, if the reaction is slow with aliphatic compounds, 3-octanone (II) and butan-2-one (V) this is mainly due to the fact, that this kind of products is not well accepted by the enzyme site. In this case, we believe that this kind of

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substrate - a prochiral ketone - with apolar substituent R1 and R2 isn't adapted with residues of the enzymatic receptor pocket, which tends to accept the aromatic and polar patterns. For example, in the case where R2 is chloroethyl: a polar group, the reduction yield is almost quantitative (see entry 6 in Table 1) after just two to three days. However, in the case where the aromatic substituent have the donors or withdrawing groups in para position, this does not alter significantly the kinetic reaction as for example when the moieties in para on the benzene are methyl or a nitro groups. [35,36, 37] In general, we can say that the kinetic reaction is rather dictated by electronics aspects than the steric considerations related to the volume of substituent. However we could not understand the slow reactivity of chlorinated substrate VI in glycerol (see entry 6 in Table 1) compared with the same essay carried out in the water.[38]. Finally, we will say, that it is difficult to understand all aspects of one complex system where many variables are involved, but understand the essential. This is understandable and consistent with the literature.[39, 40].

When the reaction is finished [41], the extraction of product from water with an organic solvent requires large amounts of solvent [42]. This method is a very expensive process. However with the glycerol the extraction is simpler and the amounts of solvent are less than with the water extraction [43]. This is reflected on the conversion that is better with the glycerol, but with the same enantioselectivity. The dilemma in this manipulation is a choice of solvent: the water is inexpensive and abundant compared to glycerol where the extraction conditions are better. [44–46]

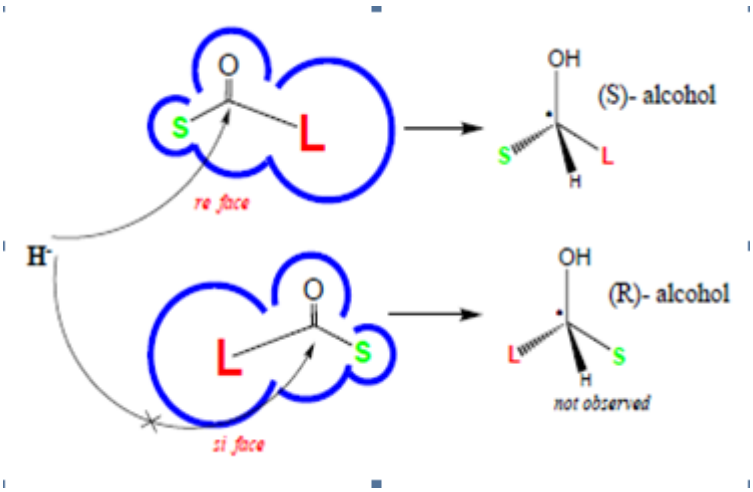

Figure 2 hydrure attack on re-face with prelog's rule.

Stereoselectivity

To understand the stereochemical trend in the enzymatic reductions [47, 48], all the products in Table 1 are drawn in such a way that the _-face (*re-face*) of the carbonyl group in the substrates is attacked by H– (NADPH) (see fig 2). The enantiomeric purity of these products was high in most cases, where the substrate is in desired structure and the Prelog's rule is confirmed (entry 1 and 4 in Table 1). Although we have previously reported that the present reductase is one of the enzymes that contribute to Prelogs rule[49, 50], the anti-Prelog-type[51, 52] alcohols are not observed in this study; the left-hand moiety is bulkier than the right-hand moiety. In second attempt the glycerol (Table 1) is used and the results are reproduced below. The assignment of the *R* and *S* configuration has been confirmed by the optical rotation and chiral chromatography. We have noted that the *R* product is more retained than the *S* product in the all attempts. This confirms the attack of the hydrure on the side Re.

CONCLUSION

In this work we wanted to show the activity of tomato (*Lycopersicum esculentum*) . That is interesting for valorization as biotechnological process for synthesis biological active secondary alcohols with *S* configuration.

If this activity is slow compared with another reductases, [63] in particular with the small prochiral ketones, when the enzymatic pocket can't accept this substrates. We could show that the selectivity is always excellent when the reaction works and respect Prelog's rules, even if it is slow. We were able to define the structure and nature of the substituents around the carbonyl, for the best yields. Thus the best substrate is one with polar substituents. We believe that the matter responsible of enzymatic activity is not very significant compared to the total mass and can be adjusted by the large quantity of tomato wastes. We have also shown that the use of glycerol can increase the yield of the reaction without being very significant. Finally we have also shown that the S-enantiomer is more retained by the chiral chromatography column and leaves the latter in all cases studied.

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