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ESCUELA DE INGENIERÍAS INDUSTRIALES

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Grado en Ingeniería Química

Extracción de curcuminoides con agua subcrítica

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TFG REALIZADO EN PROGRAMA DE INTERCAMBIO

TÍTULO: **Extraction of Curcuminuids with Subcritical Water**

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Abstract

El objetio de este TFG es determinar las mejores condiciones para la extracción de curcuminoides en la cúrcuma con agua caliente presurizada, como alternativa al método actual de extracción con etanol.

Se han realizado extracciones a diferentes tiempos (5-120 minutos) y temperaturas (100-200ºC), siendo la presión la suficiente para garantizar agua líquida. Se ha obtenido la influencia de estos parámetros en el rendimiento d extracción, habiendo sido calculado el porcentaje de masa total extraída y análisis HPLC y DPPH.

Los resultados obtenidos son un rendimiento de extracción máximo (70%) a 175ºC a cualquier intervalo de tiempo entre 5 y 120 minutos. La mayor concentración de curcuminoides en el extracto es obtenida a 100ºC y 120 min (19,53mg/g). La mayor cantidad de curcuminoides extraídos de la cúrcuma es obtenida a 125ºC y 30 minutos (10,2mg/g).

Las extracciones con mayor capacidad antioxidante fueron aquellas a 200ºC y 60 o más minutos (18% inhibición).

Palabras clave:

Extracción con agua subcrítica, cúrcuma, antioxidantes.

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Finally, to my parents: you made everything possible, thanks for all.

Abstract

The purpose of this diploma thesis is to determine the best conditions for the extraction of antioxidants in curcuma, mainly curcuminoids, with pressurized hot water, while the currently industrial way to obtain these antioxidants is the ethanol extraction. This can be a more environmental friendly way, without organic solvent.

The extractions were performed for different times between 5 minutes and two hours, and in the temperature range from 100 to 200ºC, while the was high enough to assure liquid water at this conditions. The influence of process parameters of the yield of extraction was determined. The extracts were analyzed of the content of curcuminoids by HPLC and their antioxidant activity by DPPH.

The results obtained are a maximum extraction yield at 175ºC, being practically constant in the range of temperatures performed. The highest concentration of curcuminuids in the extract has been obtained at 100ºC and 120 minutes of extraction. The extraction with the greatest amount of curcuminoids extracted per gram of curcuma has been obtained at 125ºC, after 30 min of extraction.

At temperatures of 175 and 200ºC all the curcuminoids are degraded to hydroxyl-cinmamics acids as paracumaric and ferulic acid. These substances have also a good antioxidant character; extractions with the highest antioxidant capability were those at 200ºC and 60 or 120 minutes. Higher antioxidant capability can be expected in extractions with longer times or higher temperatures.

Key words: subcritical water extraction, curcuma, antioxidants.

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Symbols

Symbol	Description	Units
MW	Molecular weight	g/mol
σ	Surface tension	N/m
B	Constant (surface tension)	N/m
b	Constant (surface tension)	[]
μ	Constant (surface tension)	[]
T_c	Critical temperature of water	K
T	Temperature	K
C_{O2}	O2 concentration of inert atmosphere	%(volume)
$C_{O2\ atm}$	O2 concentration of air	%(volume)
P_{atm}	Atmospheric pressure	bar
P_b	Blanketing pressure	Bar
P_{set}	Extraction pressure	Bar
n	number of blanketing	[]
% inhibition	Inhibition of UVA-Vis (antioxidant activity)	%
A_A	Blank absorbance	%
A_B	Sample absorbance	%
E	Extract yield	%
m_e	Extract and flask mass	g
m_f	Empty flask mass	g
m_c	Curcuma mass	g

Abbreviations

HPSCW	High pressure subcritical water
C	Curcumin
DMC	Demethoxy curcumin
BDMC	Bidemethoxy curcumin
HPLC	High performance liquid chromatography
DPPH	2,2-diphenyl-1-picrylhydrazyl
DME	Dimethoxy ethane
DMF	Dimethyl formamide
DMSO	Dymetyl sulfoxide
HMPA	Hexamethyl phosphoramide
HMPT	Hexamethyl phosphortriamide
MTBE	Methyl tert-butyl ether
NMP	N-Methyl-2-pyrrolidone
THF	Tetrahydrofuran

1.Introduction

Curcuma longa is a common plant that grow in rainy and warm regions, and his rhizomes have been used the last 4000 years as food colorant, spicy and dye for clothes and skin [1]. The most interesting kind of compounds of this rhizome, and the reason of his properties, are curcuminoids, which represent the 3-5% of the rhizome mass. These compounds are curcumin (50-60%), demethoxy-curcumin (20-30%) and bis-demethoxy-curcumin (7-20%) [2], all of them with an antioxidant, anti microbial and anti protozoan, anti proliferative, anti-angiogenic, and anti-inflammatory powerful capability. The dry and minced rhizome is used as ingredient for traditional homemade medicine in India and China [2].

Nowadays, curcuminoids are being extracted with organic solvents, mainly ethanol, due to the low solubility of curcuminoids in neutral and acid water solutions, and due to the fact that ethanol is the harmless organic solvent for human being [3].

This thesis form part of the study of alternative ways to obtain curcuminoids, as aer supercritical fluid extraction, soxhlet extraction, and ultrasonic maceration, performed by Tina Perko, Matej Ravber, Zeljko Knez, Mojca Skerget, in the Faculty of Chemistry and Chemical Engineering, Laboratory for Separation Processes and Product Design, University of Maribor.

Extraction with pressurized water is a harmless and more environmental friendly way to obtain curcuminoids, following the steps of the green chemistry of XXI century. This green chemistry means the obtainment and use of natural products instead of synthetic ones, by eco and respectful ways, with the environment, society, producers, users and handlers.

Extraction of curcuminoids with pressurized water meets all the targets above mentioned. Curcuminoids are natural compounds with interesting properties for health and welfare, and extraction with pressurized water is a non organic solvent extraction, without toxic wastes for people and environment, harmless solvent for users and handless and the high pressure industry is enough developed to be considered as safe.

The use of high pressure subcritical water, HPSCW, as solvent in extraction is a feasible candidate to obtain natural curcuminoids from turmeric due the excellent properties of pressurized water as solvent and totally innocuous at room conditions.

The purpose of this study is to determinate the feasibility of this technique and to find the best conditions for the extraction of curcuminoids with HPSCW in order to define the boundaries and optimize the work conditions of real plants in the future.

2.Theory and literature review

2.1 Curcumin, curcuminoids and curcuma

Curcumin, a bis-α,β-unsaturated β-diketone, known also as diferuloymethane [4] [2], is a natural polyphenolic compound, the main curcuminoid one, obtained in the turmeric (*curcuma longa*) rhizomes. Curcumin has interesting pharmacological properties, being anti proliferative (prevent the production of new cancerous cells), anti-angiogenic (hinder the production of new blood vessels in tumors), anti-inflammatory and antioxidant [2]; due his high yellow color, his main purpose along history has been as colorant. His code is E-100, in the group III, by the European Commission of Food and Feed safety [5].

Curcuma longa is a rhizomatous herbaceous plant, from the family of the zingiberaceae. It demands a temperature between 20-30ºC and often rainfall. It grows in the southwest of India, China and Indonesia, where has been used as medicine due the curcuminoids properties. Turmeric contains moisture (>9%), curcumin (5–6.6%), extraneous matter (<0.5% by weight), mould (<3%), and volatile oils (<3.5%). The components responsible for the aroma of turmeric are turmerone, arturmerone, and zingiberene [1].

Curcuminoids, C, are a kind of di-phenyl-heptanoids, secondary metabolites produced by plants. As his name means, di-phenyl-heptanoids, are a heptane chain with two phenyl rings in both ends, with a keto or alcoholic functional group in 3 and 7 carbons [6]. Their properties are the same as the curcuma's ones, being this just the most common of them. These curcuminoids are demethoxy-curcumin, DMC, and bis-demethoxy-curcumin, BDMC.

The main difficulty in the curcuminoids application is the difficulty of ingest due his very limited solubility in water and acid solutions as the gastric acid [7]. By this way, his bioavailability is scarce, not just in order to supply it as medicament, but allocate it in the specific tissue in effectives amounts. As additional fact, curcumin in particular has also a rapid metabolism, making this bioavailability even lower [2] [8] [9].

Nowadays new methods to improve the bioavailability are been developed, as complex with nanoparticles, liposomes, micelles and phospholipids [7]. This new methods allow a slower metabolism, increasing the permeability against the intestinal conditions, like pH and enzymes. Other way for this purpose is encapsulation [2].

Table 1: Properties of curcumin, obtained from toxnet.nlm.nih.gov database [9]

Curcumin					
Molecular Formula:	C21-H20-O6	[10]	Molecular Weight:	368.39	[10]
Color/Form: Orange-yellow, crystal powder; gives brownish-red color with alkali; light-yellow color with acids		[10]			
the crystal powder is Orange yellow prisms, rhombic prisms from methanol		[11]			
Melting Point:	183ºC	[10]	Vapor Pressure(25ºC):	$3.08 \cdot 10^{-12}$ mmHg (est)	[12]
Spectral Properties: MAX ABSORPTION (DIOXANE):			265 NM (LOG E= 4.18) 420 NM (LOG E= 4.77)		[13]
MASS: 75938 [11]	IR: 13460 [11]		UV: 6-924 [11]		
Solubility:					
In water(25ºC):	3.12 mg/L(est)	[12]	Insoluble in ether		[10]
Soluble in glac. acetic acid		[10]	Soluble in alcohol		[10]
Octanol/Water Partition Coefficient:			log Kow = 3.29 (est)		[14]
Henry's Law constant (25ºC):			7.04X10-22 atm m3/mol (est)		[12]
Other Chemical/Physical Properties:					
Specific gravity (15ºC):	0.982-1.01	[15]	refractive index (20ºC):	1.5023-1.5088	[15]
optical rotation (90% ethanol, 10% turmerone):			+8º - +17º		[15]
Slightly fluorescent		[16]	acid value:	0.3-2.4	[15]
ester value:	16-22.4	[15]	ester value (after acetylation):	56-73.4	[15]

2.2 Solid liquid extraction

Solid liquid extraction, or leaching, is the separation process of a substance originally in a solid matrix by another, based on the capability of dilute the first one, called solute, in the second one, called solvent. This solvent must be selective, to dissolve just the substances target. After the extraction, there are two phases, one is the solution of solute in the solvent, this is called extract; the other phase is the solid phase, this one often is not dry, being wet by the extract, the name of this wet solid phase is waste.

To perform the extraction, it's required:

Good contact between the solid and solvent phase,

Two different and separable phases.

The factors that determine the amount of solute extracted can be about steady or transitory process. Being the first ones:

-the solubility equilibrium between solute and solvent,

-the liquid kept by the solid in the phases separation process.

The transitory factors are all the mass transfer process:

-diffusion of solvent in solid,

-dissolution of the solute in the solvent,

-diffusion of solute in solvent through solid, and finally,

-diffusion of solute in solvent out the solid particle.

In order to increase mass transfer, the particle's size must be as small as it's possible, to enable short times of diffusion throw the solid; and the extraction process under agitation conditions, to improve the diffusion of the solute in the solvent. The solution process is just function of chemical parameters that cannot be improved changing mechanic properties.

With enough agitation and particles smallness, the extraction process can be considered stationary, being the yield ratio just function of temperature, that changes dissolution properties as transitory speed process and saturation capacity of the solvent. In this experience, this affirmation is not true due the reaction process, as it will be explained later.

2.3 Extraction of natural products

2.3.1 History:

Along history, different methods have been developed to obtain natural products from natural raw materials. Extraction of natural compounds started in Egypt and Mesopotamia during the ancient history, looking for compounds to produce high value products as pharmaceutical oils, waxes and perfumes, being the oldest evidence obtained dates 3500BC. Lately, several Sumerian texts (2100BC) show technological knowledge in extraction. This develops use water as solvent and inorganic additives, in order to change the water properties [17].

The first evidence of extraction with organic substances dates 1600BC, being used beer and wine as solvent. But the real development of this field started in 900AC, once ethanol was obtained in enough high concentrations [17].

2.3.2 Methods of extraction:

There are different ways of extraction, in order to the properties of the substance to be extracted.

Maceration:

The easiest extraction is supply contact between solvent and the particles where the solute is placed, keeping it together the needed time. This process can be with or without agitation. The particles must be pulverized, in order to increase the surface per mass ratio, being transferred the solute to the solvent.

The container can be emptied and filled with fresh solvent, to supply more capacity, if the previous one was saturated, or just to reduce the concentration of solute in the solvent kept by the waste, being a crosscurrent process. This process can be done too using the extract of this step as solvent in the previous one, being now countercurrent.

As last step it's needed a way to separate the waste and the extract, for this purpose the industrial ways are filtration, mechanic press or centrifuge.

Due his easy mechanism, maceration was the first extraction method executed.

According the experiments performed by T. Perko et Al. [3] the extraction yield of 6 hours maceration of 10g curcuma with 250 mL ethanol at room temperature is 10.40%, being the amount of curcuminoids 166.45 mg curcumin (C), 84.35 mg demethoxy curcumin (DMC) 100.51 mg bis-demethoxy curcumin (BDMC), 351.32 mg curcuminoids per extract gram, and his antioxidant capability is 34%.

Extraction with solvent reflux:

The main feature of extraction is that remove the solute from one substance to another so, obviously, this new solution must have an easier way to be separated in his compounds. If boiling is the way, the solution can be evaporated in order to obtain fresh solvent, to be used again after being condensed. This way of work allows a small use of solvent per solute extracted. Extraction with soxhlet can be taken as example of extraction with solvent reflux.

The results obtained with soxhlet [3] in the same conditions (6h at room temperature, 10g curcuma in 250 mL ethanol) shows a raising of extraction, composition and antioxidant capability: 161.27 mg C, 111.04 mg DMC and 125.24 mg BDMC: 397.55 mg curcuminoids. Being the inhibition value 52%.

Percolation:

The process of fluids moving throw porous solids, also called percolation, is a way to produce the contact between the solvent, as fluid, and the solute in the solid. As industrial method, the solid is placed on a filter and sustained by gravity, or fixed between two. The feed of solvent use to be fresh, in the inlet of the filter and it gets solute as it passes throw the solid, may being in equilibrium in part of the thickness. As the fluid flow, the inlet gets empty of solute, so the equilibrium is done with the next particles, until all the solvent has been removed from the entire solid. The easiest example for this process is a coffee machine.

Percolation has not been studied as method to extract curcuminoids.

Ultrasound maceration:

As method for small extractions, as essential oils extraction, ultrasound can be a support in maceration. High resonance mechanic waves produce cavitation, due the fast change of

pressure. This cavitation produces the rupture of the cell wall. The cavitation produces bubbles just in contact with the solid, being an implicit mixer that increases the mass transfer in the place of maximum concentration. The ultrasonic produces also an increasing of the temperature, fact that raises the solubility of the solute in the solvent [18].

Maceration of curcuma supported with ultrasounds, has been performed by Perko et Al. [3], being used 1 gram of curcuma and 25 mL of Ethanol. The maceration takes 30 minutes, at 20ºC, being the ultrasound fixed at 400W. The results obtained are 13.44 mg C, 69.44 mg DMC and 14.63 mg BDMC: 97.51 mg curcuminoids per gram of extract, and the absorption, around 20%.

Microwave maceration:

The use of microwave energy gives to the maceration the chance to allocate the energy in specific places, the organic compound and the cell wall. This allocated heat produces a dilatation of the cell, and his wall rupture. Once the cell is broken, the solvent gets inside, being heated by the cell itself in a mass and heat diffusion mechanism. This bigger temperature of the solvent raises the solubility of the solute in the solvent [18].

There are not studies of microwave maceration of curcuma due the good solubility of this one in ethanol at room temperature.

Chromatography:

Based in a multi infinitesimal stage distribution due the different affinity of each extract substance with the static and mobile phases, withhold and dragged respectively, chromatography reaches the total separation of the solute after enough time or length. Chromatography is used as analytic tool, qualitative and quantitative, not as separation process due his low mass capacity of operation. By this way chromatography has not been studied for curcuminoids procurement, but has been used for analysis study after all the experiments.

Supercritical fluids extraction:

Supercritical fluid is a fluid with a pressure and temperature higher than his critical point. In these conditions the different liquid and vapor phases doesn't exist. This fluid has particular properties, having his diffusion coefficient the same orders as his liquid phase, and his viscosity

the same order as his gas phase. But the most interesting property of supercritical fluids is the high reliance of solubility with density and this one with pressure and temperature [19] [20]. The fact of dispose solubility as controllable variable has especial interest in extraction process. The most common fluids for this kind of extraction are CO_2 and water.

Several experiments, using CO_2as solvent, have taken place [3], being the best results obtained at 60ºC and 200 bars: 18.88 mg C, 2.70 mg DMC and 5.03 mg BDMC: 26.60 mg of curcuminoids per gram of extract. This result reveals this method is not the best for curcumin extraction. The absorption is around 24%.

2.3.3 Solvent:

Speaking about natural products, there are a lot of restrictions, due the final purpose of the extract, being this often nutritional. The most common because his abundance, nonexistent hazard and usability, has been water, perfect to extract polarity compounds. To extract non polarity compounds can be used foodstuff desirably, or other organic solvents like propene, butane, acetylene, CO2, ethanol, etc. The rule “like extracts like” must be used as first key to choose the solvent, according to Van Laar theory [21], but solution solubility can be improved using mixtures [22].

Water or foodstuff residue in final extract is assumed to be harmless, for the rest of organic solvents their amount is limited in order not just to guarantee quality but safety.

The most common organic solvents and their solubility properties are shown in the table 2 [23]:

Table 2: Properties of organic solvents, compiled by Steve Murov [23]

Solvent	formula	MW	boiling point (°C)	melting point (°C)	density (g/mL)	solubility in water (g/100g)	Dielectric Constant	flash point (°C)
acetic acid	$C_2H_4O_2$	60.052	118	16.6	10.446	Miscible	6.20	39
acetone	C_3H_6O	58.079	56.05	-94.7	0.7845	Miscible	21.01	-20
acetonitrile	C_2H_3N	41.052	81.65	-43.8	0.7857	Miscible	36.64	6
benzene	C_6H_6	78.11	80.1	5.5	0.8765	0.18	2.28	-11
1-butanol	$C_4H_{10}O$	74.12	117.7	-88.6	0.8095	6.3	17.8	37
2-butanol	$C_4H_{10}O$	74.12	99.5	-88.5	0.8063	15	17.26	24
2-butanone	C_4H_8O	72.11	79.6	-86.6	0.7999	25.6	18.6	-9
t-butyl alcohol	$C_4H_{10}O$	74.12	82.4	25.7	0.7887	Miscible	12.5	11
carbon tetrachloride	CCl_4	153.82	76.8	-22.6	1.594	0.08	2.24	--
chlorobenzene	C_6H_5Cl	112.56	131.7	-45.3	11.058	0.05	5.69	28
chloroform	$CHCl_3$	119.38	61.2	-63.4	14.788	0.795	4.81	--
cyclohexane	C_6H_{12}	84.16	80.7	6.6	0.7739	<0.1	2.02	-20
1,2-dichloroethane	$C_2H_4Cl_2$	98.96	83.5	-35.7	1.245	0.861	10.42	13
diethylene glycol	$C_4H_{10}O_3$	106.12	246	-10	11.197	10	31.8	124
diethyl ether	$C_4H_{10}O$	74.12	34.5	-116.2	0.713	7.5	4.267	-45
diglyme	$C_6H_{14}O_3$	134.17	162	-68	0.943	Miscible	7.23	67
(glyme, DME)	$C_4H_{10}O_2$	90.12	84.5	-69.2	0.8637	Miscible	7.3	-2
(DMF)	C_3H_7NO	73.09	153	-60.48	0.9445	Miscible	38.25	58
(DMSO)	C_2H_6OS	78.13	189	18.4	1.092	25.3	47	95
1,4-dioxane	$C_4H_8O_2$	88.11	101.1	11.8	1.033	Miscible	2.21(25)	12
ethanol	C_2H_6O	46.07	78.5	-114.1	0.789	Miscible	24.6	13
ethyl acetate	$C_4H_8O_2$	88.11	77	-83.6	0.895	8.7	6(25)	-4
ethylene glycol	$C_2H_6O_2$	62.07	195	-13	1.115	Miscible	37.7	111
glycerin	$C_3H_8O_3$	92.09	290	17.8	1.261	Miscible	42.5	160

Table 3: Properties of organic solvents (continuation)

Solvent	formula	MW	boiling point (°C)	melting point (°C)	density (g/mL)	solubility in water (g/100g)	Dielectric Constant	flash point (°C)
heptane	C_7H_{16}	100.20	98	-90.6	0.684	0.01	1.92	-4
(HMPA)	$C_6H_{18}N_3OP$	179.20	232.5	7.2	1.03	Miscible	31.3	105
(HMPT)	$C_6H_{18}N_3P$	163.20	150	-44	0.898	Miscible	??	26
hexane	C_6H_{14}	86.18	69	-95	0.659	0.014	1.89	-22
methanol	CH_4O	32.04	64.6	-98	0.791	Miscible	32.6(25)	12
(MTBE)	$C_5H_{12}O$	88.15	55.2	-109	0.741	5.1	??	-28
methylene chloride	CH_2Cl_2	84.93	39.8	-96.7	1.326	1.32	9.08	1.6
(NMP)	CH_5H_9NO	99.13	202	-24	1.033	10	32	91
nitromethane	CH_3NO_2	61.04	101.2	-29	1.382	9.50	35.9	35
pentane	C_5H_{12}	72.15	36.1	-129.7	0.626	0.04	1.84	-49
ligroine	--	--	30-60	-40	0.656	--	--	-30
1-propanol	C_3H_8O	88.15	97	-126	0.803	Miscible	20.1(25)	15
2-propanol	C_3H_8O	88.15	82.4	-88.5	0.785	Miscible	18.3(25)	12
pyridine	C_5H_5N	79.10	115.2	-41.6	0.982	Miscible	12.3(25)	17
(THF)	C_4H_8O	72.106	65	-108.4	0.8833	30	7.52	-14
toluene	C_7H_8	92.14	110.6	-93	0.867	0.05	2.38(25)	4
triethyl amine	$C_6H_{15}N$	101.19	88.9	-114.7	0.728	0.02	2.4	-11
water	H_2O	18.02	100.00	0.00	0.998	--	78.54	--
water, heavy	D_2O	20.03	101.3	4	1.107	Miscible	??	--
o-xylene	C_8H_{10}	106.17	144	-25.2	0.897	Insoluble	2.57	32
m-xylene	C_8H_{10}	106.17	139.1	-47.8	0.868	Insoluble	2.37	27
p-xylene	C_8H_{10}	106.17	138.4	13.3	0.861	Insoluble	2.27	27

In order to choose the best solvent, there are basic rules:

-selectivity: a high selectivity for the compound target is desired, by this way it's not necessary more process after or before the extraction.

-capacity: the amount of solute in solvent is a important factor mainly due to reduce the amount of solvent needed to extract the solute, but in order to allow a fast extraction too, increasing the difference between the solvent an solid phase, and by this way, increasing the speed also.

-chemically inert: the reaction between the solvent and solute is not desired, because reduce the amount of both of them and produce undesired byproducts. The reaction between solvent and waste is not desired too, because new molecules produce undesired changes properties of the solution.

-highly volatile: in separation process the target is to remove determined compounds from a current, and have it in other. The extraction process just produce the first step, to make the second one it is needed a way to separate solvent and solute definitely. For this purpose evaporation is desired, being demanded for this a high difference between the volatility of both compounds.

-harmless.

-environment friendly.

-as cheap as possible: where price doesn't means just the price of the solvent but too all the features mentioned before: cost of the extraction process, cost of the separation, insurances, patents, licenses, fines…

2.4 High pressure water extraction

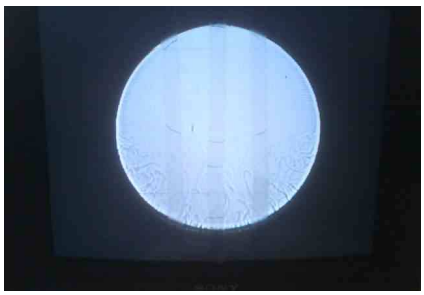


figure 1: water at critical point. Udo Van Hes [23]

Pressurized water is water with a pressure between 1 and 218 bars being the critical point at this pressure, and 374 º C temperatures [25]. A higher pressure and temperature, water stays as supercritical fluid, this doesn't fits in the target of the study.

Water as solvent has a very particular properties. At room temperature, water has a disproportionately high boiling pressure, high dielectric constant and polarity according to his own mass. The cause of this is the hydrogen bonded structure has a relative very high strength (5-6 Kcal/mol) due the high polarity density of the hydrogen atom. This strength is of course smaller than molecular bonding (being around 5% of the weakest bond), but it's much bigger than the Van der Waals ones [26].

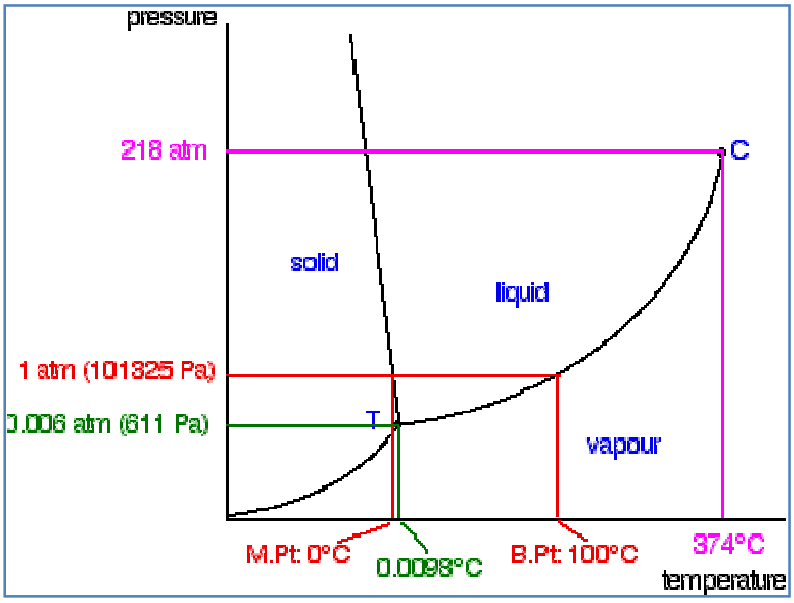


Figure 2: Location of triple and critical points of water. [27]

These properties that define the solvent, and others, change along the temperature goes up, as follows.

2.4.1 Pressure:

First of all, as it's shown in [25], for biological extractions, pressure can supply mechanic strength to broke cells, but, being enough to guarantee the liquid phase of the solvent, pressure is not a factor in the chemical properties of the solvent.

In an isochoric saturated system, the pressure is function of temperature, as is shown in figure 3 [28]:

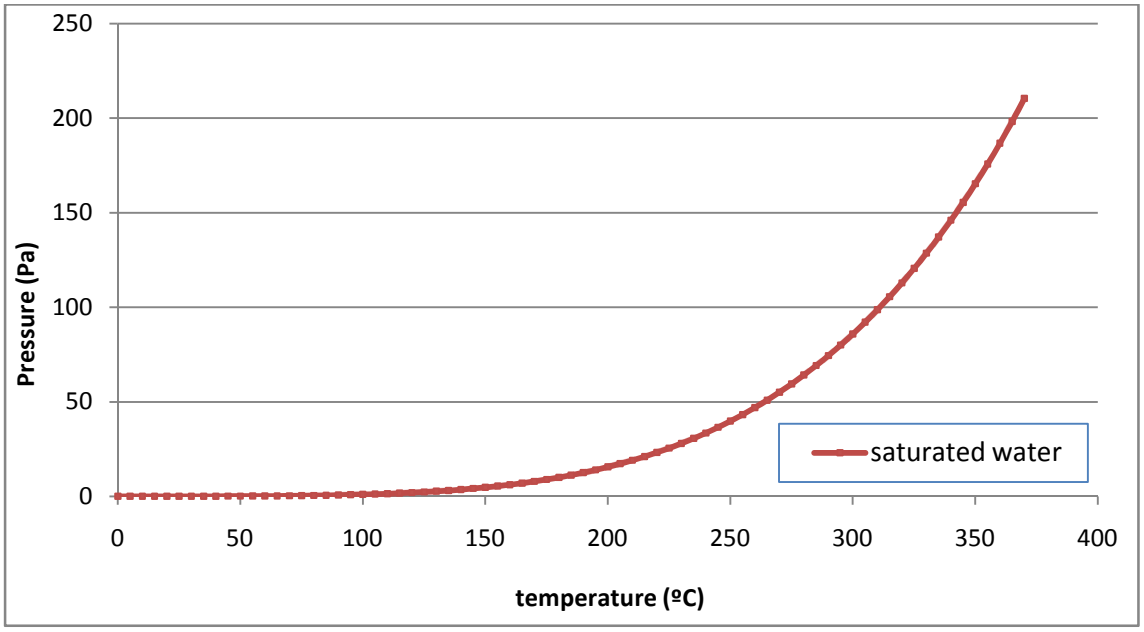


figure 3: temperature/pressure relation of saturated water as fluids definition, calculated by NIST webbook [28]

The relation between pressure and temperature lacks of significance in chemical and physical properties for the extraction results, but the combination of both defines the properties of the sample and the ambient work. In an isochoric system, as the one used in this study, the control of one (Temperature) entail the change of the second one (pressure).

2.4.2 Density:

The increasing of the temperature of a liquid fluid implies the uprising of the movement of his molecules. This agitation supplies more empty space between molecules, fact that reduce the density.

The same process of the agitation because of heat that accelerate the movement of molecules, increasing the diffusion, gives also more free place to the free circulation of these particles. By this way, an increasing of density of the liquid due a higher temperature produces an increasing of mass diffusion coefficient.

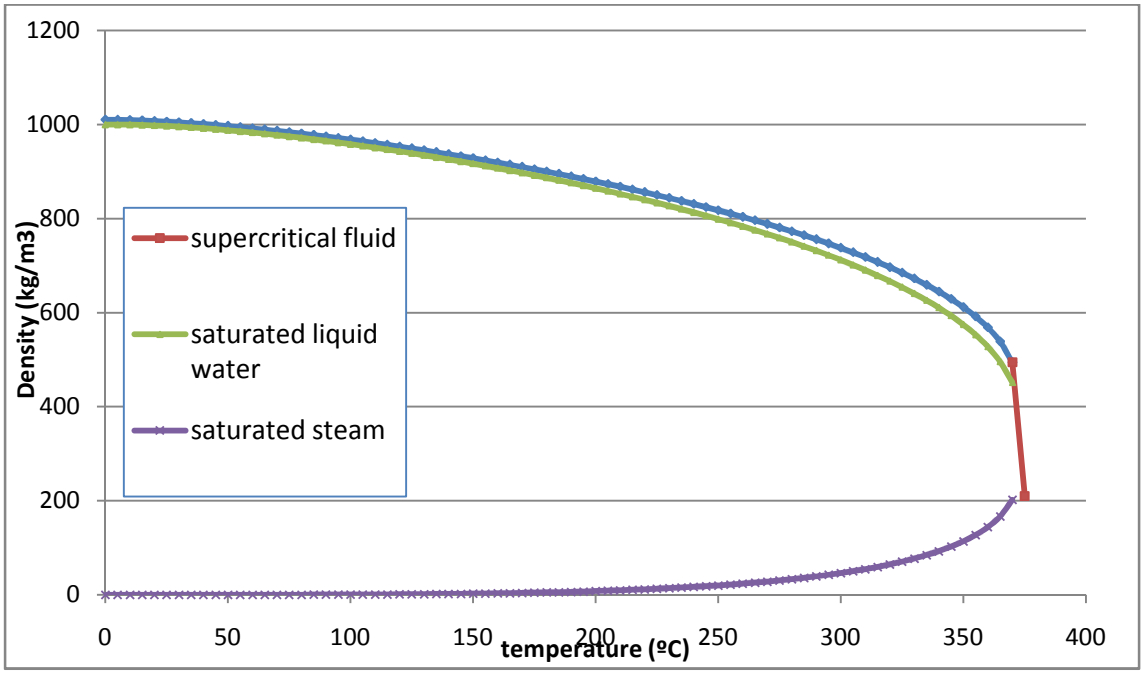


Figure 4: density of saturated liquid and gas water as fluid definition, calculated by NIST webbook [28]

Density of liquid water decreases slowly, until the evaporation begins. If the pressure is fixed enough high to guarantee the liquid phase of the solvent until the critic temperature, all this range can be used, being the density at these conditions (374ºC & 218 bar) 494.53 kg/m3, 48.93% density at 0ºC, 218 bar [28].

The density of liquid water variation along the temperature is relevant, but stay at the same order of magnitude.

2.4.3 Viscosity:

Viscosity has very relevant properties in diffusion process. The coefficient between viscous diffusion rate and mass diffusivity is the non-dimensional Schmidt number. Between gases, this number always round the unit, this doesn't happen with liquids.

The reduction of viscosity appoint the reduction of the energy implied in the penetration of the solvent in the solid and the output of the solute, taking the diffusion process less time in be completed, and having faster mass transfer process[29].

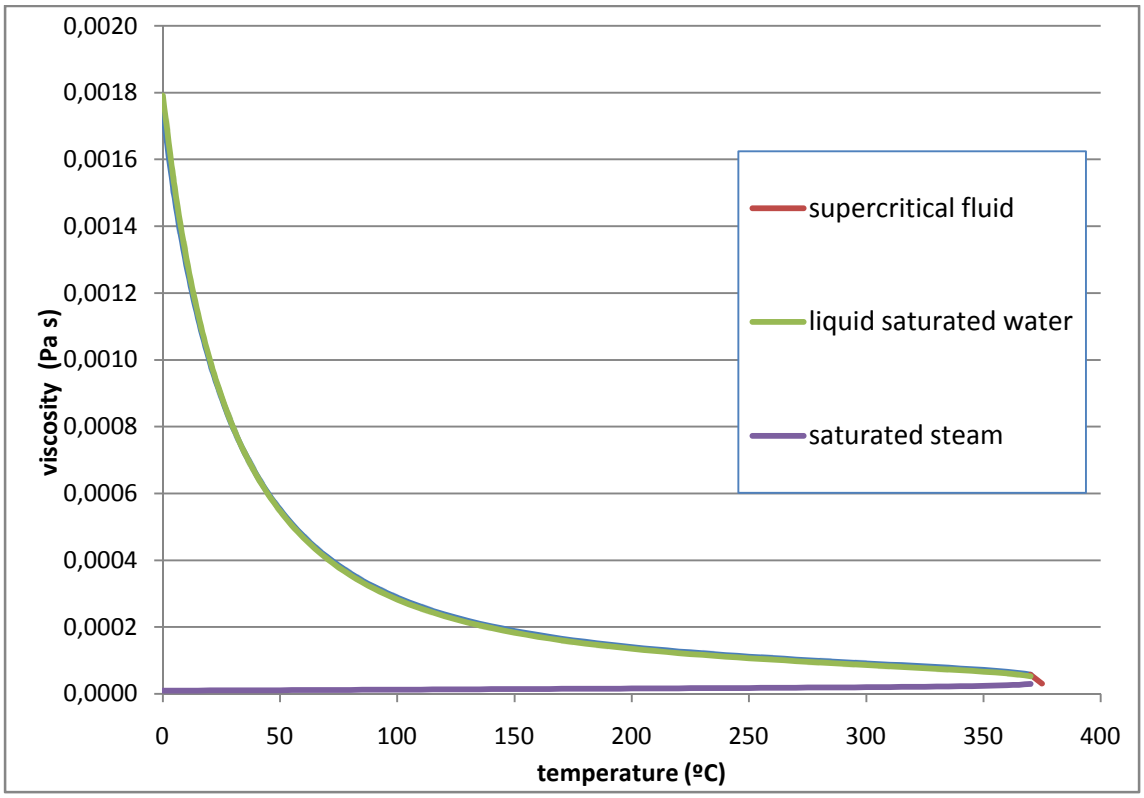


Figure 5: viscosity of saturated liquid and gas water as fluids definition, calculated by NIST webbook [28]

Viscosity of liquid water decreases until being close to 0 at 373ºC. This decreasing goes faster at colder temperatures. The change of this property is about several orders of magnitude, and produces an important improvement of the mechanic features of the fluid in diffusion scales.

Viscosity of saturated steam can be considered null.

2.4.4 Surface tension:

Surface tension means energy needed to increase the area of a fixed volume. The fact of cross this surface implies break it and by this way an increment of the surface is needed. So great values of surface tension will hinder transfer process.

The surface tension is modified also by the temperature, following, in saturated conditions, the equation 1 [30]:

$$\sigma = B\left[\frac{T_c - T}{T_c}\right]^{\mu}\left[1 + b\frac{T_c - T}{T_c}\right] \qquad 1$$

Being B=235.8E-3N/m; b=-0.625; μ=1.256. And all temperatures in Kelvin scale.

By this way, the relation between surface tension and temperature is represented as follows:

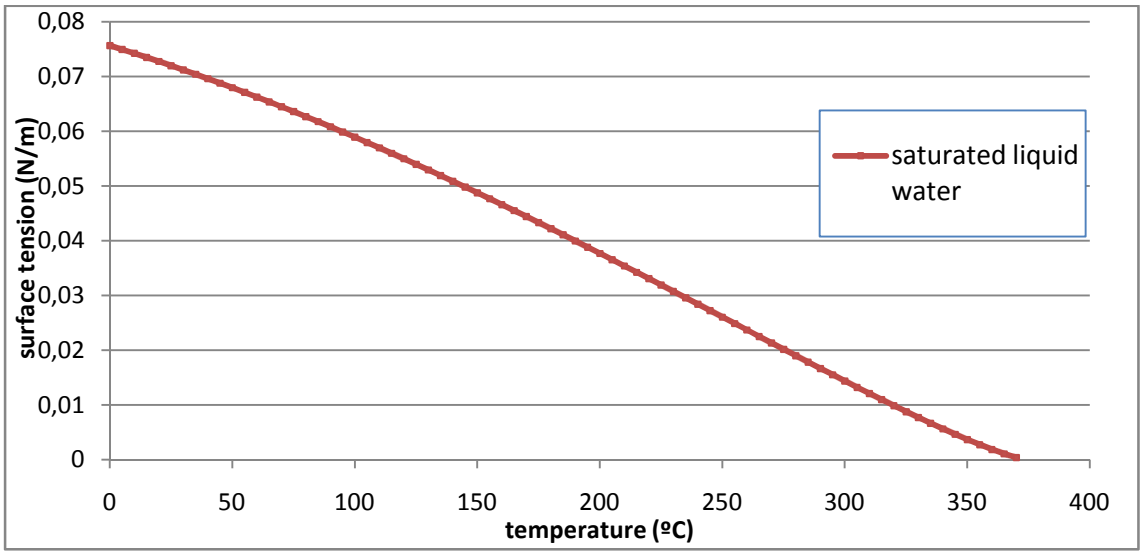


Figure 6: surface tension of saturated liquid water, calculated with equation 1.

Surface tension decreases quasi-lineally from 0.75 to zero in the critical point for saturated liquid water.

Surface tension of gasses or supercritical fluids do not exist because their total diffusivity that define them inability the occurrence of surfaces.

2.4.5 Dielectric constant:

The dielectric constant, or permittivity, is function of temperature and pressure, as was studied in [31]. In the figure 6 it's shown the dielectric constant of saturated steam and liquid water.

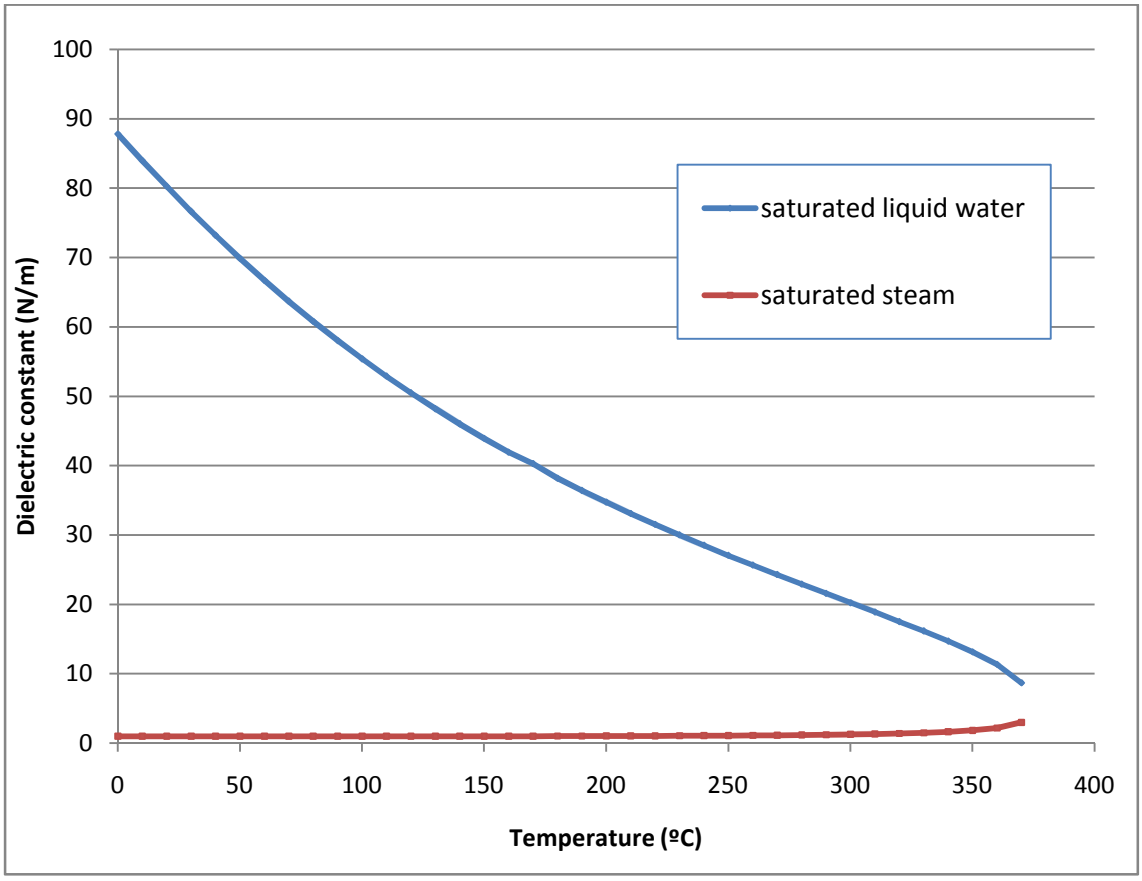


Figure 7: dielectric constant of saturated liquid and gas water, performed by NIST webbook [28]

The dielectric constant decreases in liquids while temperature increases because the random spin motion given by thermal energy grows, and conceals partially the dipole moment [32]. The value of the dielectric constant defines the kind of solutes that are best dissolved, being a measurement of his polarity.

In the other hand, the dielectric constant of the saturated steam grows sparingly, from 1 to 3, and can be considered constant.

2.5 Chemical reactions in high pressure water

The purpose of these experiments is to study the allowance of pressurized water as solvent in the curcuminoids extraction from curcuma. As has been shown before, one of the most important features in the solvent selection is the lack of undesired reactions, which really happen in water at these temperatures.

First of all, consider the molecular properties of curcumin, demethoxy curcumin and bis-demethoxy curcumin will be done.

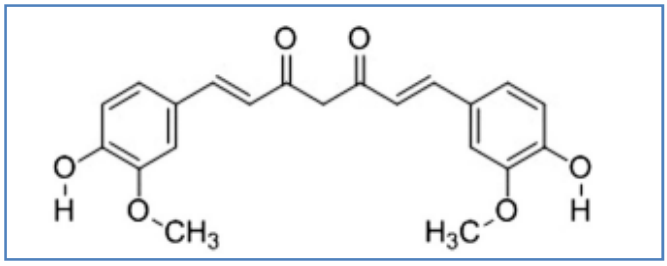


figure 8: diketo-curcumin isomer. Y. Manolova [33]

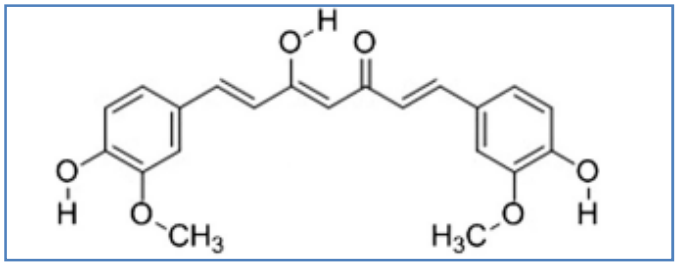


figure 10: keto-eno-curcumin isomer. Y. Manolova [33]

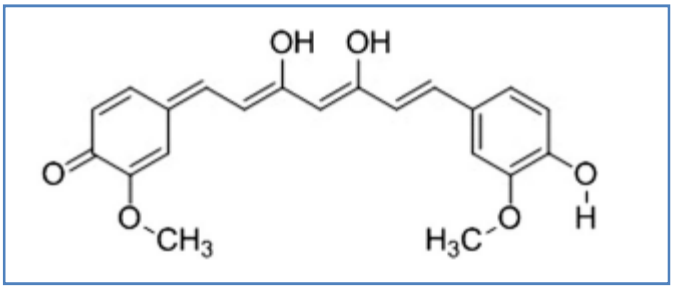


figure 9: dieno-cucumin isomer. Y. Manolova [33]

Curcumin are two aromatic rings, phenyl compounds, connected by two α,β-unsaturated carbonyl groups. This can be assumed as the union of two ferulic acid molecules by their acidic group. This union means a β-dihydroxycarbonyl that has the tautomeric reaction of aldol system. By this way, three compounds can exist together in equilibrium, keto-enol-curcumin, diketo-curcumin, and dienol-curcumin.

The last one really doesn't exist, and the balance between the first ones is mainly keto-enol phase, inside solid matrix and in as solution. The ratio of diketo phase can be raised in methanol/water solution as has been studied by Y. Manolova et al [33] (Figure 11).

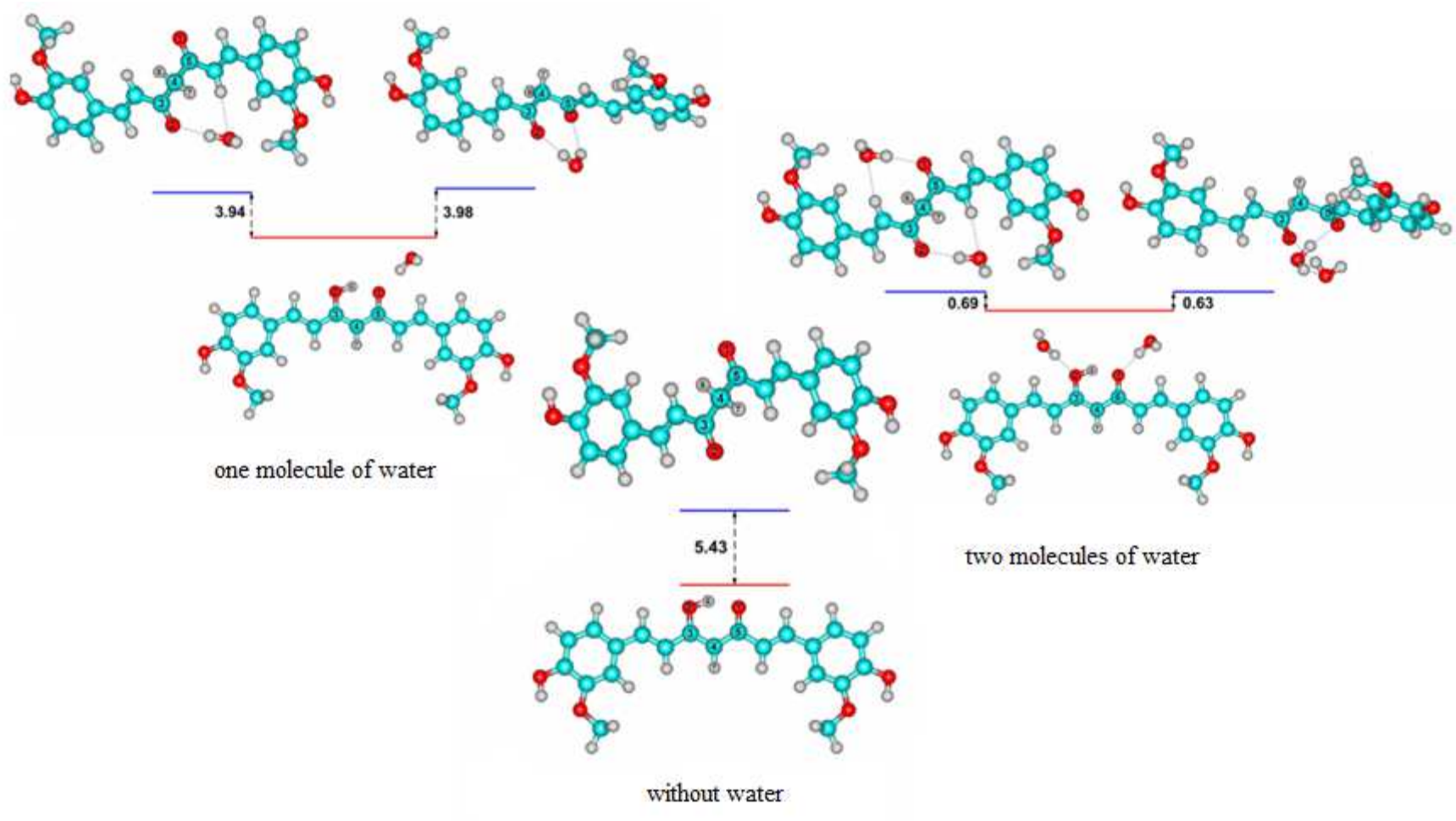


Figure 11: comparison of diketo and keto-enol curcumin isomers behavior with water molecules in methanol solution. AE calculated by Y. Manolova et Al. [33]

By this way, the solubility of curcuminoids is function of his inherent tautomeric reaction.

As has been compiled by Nermin Simsek Kus [9], the reactions that can take place in organic compounds submitted to pressurized hot water embraces almost all the organic reaction, being obviously function of the substance studied. Examples of these reactions are alkylation, condensation, coupling reactions, cyclization, decomposition, decarboxylation, dies-alder reaction, disproportionation, elimination, ene reaction, isomerization, hydrogenation-dehydrogenation, hydrolysis, oxidation, organometalic reactions and rearrangement. All these reactions can take place in specific conditions in pressurized water, which have to been fixed to arrange the optimal conditions in his production.

The lowest kinetic condition are being looked for in this experience. There are reactions that can't take place due the nonexistence of some of the reactives needed, as ene-reaction, dies-alder reaction, oxidation, coupling reactions, hydrogenation, or organometalic reactions. Others reactions won't take place due the needle of catalyst, as cyclization, dehydrogenation and hydrogenation.

Reactions that are just function of physic properties in the samples performed are alkylation, condensation, decomposition, decarboxylation, disproportionation, elimination, hydrolysis and rearrangement.

As it will be show, at high temperatures there are not curcuminoids compounds, but the antioxidant capability, issued by the phenolyc ring is bigger than samples at lower temperatures, emerging ferulic acid, paracumaric acid, 4-hydroxybenzaldehyde and vanillin. is also observable that the amount of demethoxy and bis-demethoxy compounds is bigger than curcumin along total amount of curcuminoids increases. It is reasonable understand that there are taking place reactions of hydrolysis, producing the disruption of the aldol group and the methoxy substituent, between others.

Extraction yield of specific compounds is function of the solubility of each compound in the solvent and additional facts, as common-ion effect and chelation in coordination complex, but the amount of solute is bounded by solubility. The reaction of the obtained compounds in others, obtained indirectly, with close properties can mean an increasing of these properties due the overshoot of the solubility, which still notes the limit of each component. If the solubility of the solvent fixes the limit, an increasing of the antioxidant properties of the solution can be obtained hydrolyzing the curcuminoids of a first extraction and performing a second one with a new sample. The interest of the studies is find the biggest amount of curcuminoids and

antioxidant capability per mass of solid, so the limits are really fixed by the solid, the mass transfer and the kinetic, being the solid-solvent ratio reduced if the saturation is accomplished, so this second addition of solid doesn't have interest.

Properties of interesting compounds existing in the samples after extraction are compiled in Table 4 and defined in figures 12-15:

Table 4: physical properties of products of curcuminoids decomposition. Compiled from TOXNET [9] and PUBCHEM [34] databases.

Physical Property	Units	vanillin	p-coumaric	ferulic acid	4-Hydroxy-benzaldehyde
molecular weigh	g/mol	152.1473	164.15802	194.184	124.38
molecular formula	(none)	C8H8O3	C9H8O3	C10H10O4	C7H6O2
Melting Point (760 mmHg)	ºC	81.5	211.5	---	117
Boiling Point (760 mmHg)	ºC	285	---	---	310
pKa Dissociation Constant (25ºC)	(none)	7	4.64	4.58	---
log P (octanol-water)	(none)	1,21	1.79	---	1.35
Water Solubility (25ºC)	mg/L	1.10E+03	1.83E+03	5970	8450
Vapor Pressure (25ºC)	mm Hg	1.18E-05	1.61E-05	2.69E-06	---
Henry's Law Constant (25º)	atm-m3/mole	2.15E-09	1.35E-12	7.96E-14	---
Atmospheric OH Rate Constant (ºC)	cm3/molecule-sec	2.73E-11	5.17E-11	4.83E-11	---

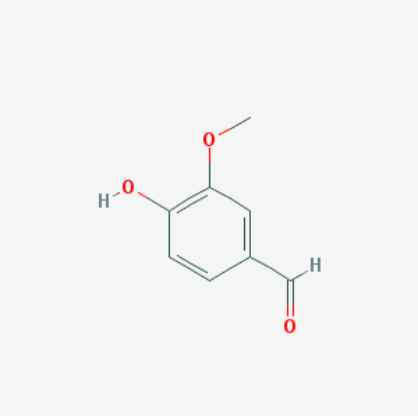


figure 13: vanilin [9]

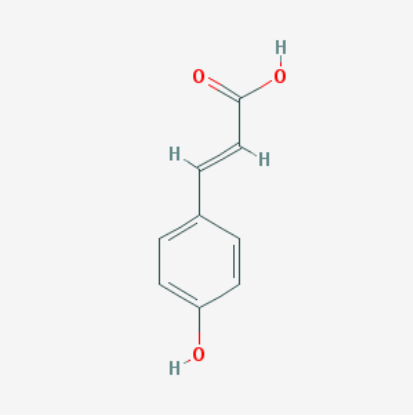


figure 12: ferulic acid [9]

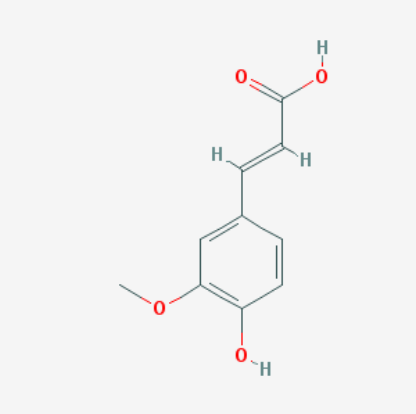


figure 14: p-coumaric acid [9]

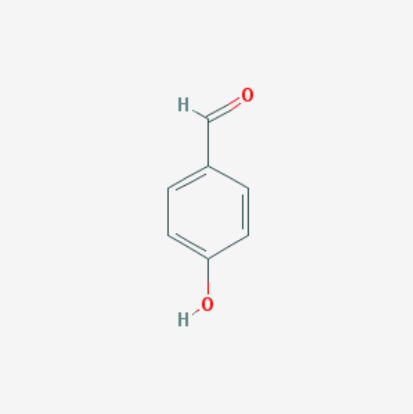


figure 15: 4-hidroxy-benzaldehyde [9]

3. Procedure

The aim of the work was to find the best time and temperature conditions for the extraction of curcuminoids compounds from curcuma. The ratio has been fixed for all experiments to 1:20 (g:mL). The autoclave was loaded with 1.5g of curcuma and 30mL of milli-Q water.

25 experiments have been performed. The pressure was set initially to 20 bars at room temperature with pressurized nitrogen, and then raised along temperature raises. Experiments have been performed changing the temperature, at 100, 125, 150, 175 and 200ºC and time of extraction, 5, 10, 30, 60 and 120 minutes. The measurements obtained during the extraction procedure have been the solid mass, the empty flask mass, the flask with the solute mass. With these values the extraction yield can be obtained. The analyses carried out have been HPLC and DPPH antioxidant activity.

3.1 Material and equipment

The equipment used has been a high pressure (400 bar) high temperature (400ºC) reactor and valves (Autoclave Engineers), manometer (Wika), thermometer (-50ºC to 1150ºC) (Greisenger), HPLC (Agilent 1100 HPL), HPLC column Agilent Eclipse XDB-C18 (150 mm × 4.6 mm; 5mm particle size), UV-Vis spectrophotometer (Varian 50 Probe).

Material used in these experiments:

Curcuma comes from Hisa Zacimb, Ljubljana, being imported from India; milli-Q water has been produced in the laboratory, dymetyl sulfoxide (Merck KGaA), acetonitryl (Sigma Aldrich), methanol (Sigma Aldrich) and acetic acid (Sigma Aldrich).

3.2 Experimental Procedure

1.5 grams of curcuma are weighed on a digital scale, and loaded in the autoclave used as extractor. 30 mL of milli-Q water are also inserted in the extractor. Once the magnetic stirrer is introduced, the autoclave is ready to be closed and heated by heating jacket. In order to avoid oxidation, oxygen is removed from the extractor loading pressurized nitrogen three times until 50 bar each one and releasing the pressure. Once the atmosphere gets inert, pressure is established with nitrogen at 20 bars. The amount of oxygen inside the extractor atmosphere is:

$$C_{O2} = C_{O2atm} \times \left(\frac{P_{atm}}{P_b}\right)^n \times \frac{P_{atm}}{P_{set}} \times 100 \cong 0.00001\% \qquad 2$$

figure 16: autoclave used in the experiments

Being $C_{O2\,atm}$ the amount of atmosphere oxygen (% volume), P_{atm} the atmosphere pressure (bar), P_b the pressure reached in the blanketing process, n the times the autoclave has been loaded, and P_{set} the pressure fixed in the extractions.

At these conditions the autoclave is heated to desired temperature, where it stays the required time, and then it is cooled with water until 30ºC.

Afterwards the sample is filtrated with a vacuum system, obtaining a yellow/red/brown liquid and a darker solid waste dependent on the temperature and time applied. Existence of colloids can hinder the filtration process; these colloids appear at 150 and 175ºC. To avoid this, the sample is centrifugated previously and, by this way, the main amount of solids is removed from the suspension, colloid gets precipitated and liquid gets diluted, so the filtration is easier.

The extract is introduced in a vacuum evaporator that is initially set at 50 mBar, being this pressure enough to evaporate water without bubbles that drag the extract and lately, when there is not possibility of bubbles, at 30 mBar until the extract is absolutely dry. Therefore water is absolutely removed, obtaining at the end the dry solute in the spherical flask, which has been weighed before. The difference between the empty flask and flask with solute is the amount of

solute extracted from the curcuma treated. The content of curcuminoids in extract and antioxidant activity of extract was analyzed by different methods, described in the following subchapter.

Samples are stored in freezer until the analysis.

3.3 Analytical procedures

Two kinds of analysis have been done, a DPPH antioxidant activity essay, and HPLC.

3.3.1 HPLC analysis of curcuminoids

20 mg of solute are added in 10 mL flask, and then are diluted with dymethyl sulfoxide, DMSO, due the presence of starch in the solute, insoluble in water or methanol at room temperature.

The analytical procedure has been directly taken from T. Perko et al, (2015) Isolation, characterization and formulation of curcuminoids and in vitro release study of the encapsulated particles. *The Journal of Supercritical Fluids*, 103, pp.48-54. [3]:

"The extracts were analyzed by HPLC, using the method described by Lee and Choung:

The Agilent 1100 HPLC sys-tem consisted of a binary pump, column heater, auto sampler and variable wavelength detector (VWD). The separation was achieved on chromatographic column Agilent Eclipse XDB-C18 (150 mm × 4.6 mm; 5mm particle size). The mobile phases were 2% acetic acid in water (elution A) and 2% acetic acid in acetonitrile (elution B). The solvent gradient was as follows: 0–3 min, 10% B;8 min, 20% B; 13 min, 25% B; 18 min, 35% B; 28–33 min, 55% B and then held for 3 min before returning to initial conditions. The solvent flow rate was 1.0 mL/min and the column temperature was30◦C. The volume of injection was 10 µL and peaks were monitored at 420 nm. Quantification of single curcuminoids was done using calibration curves obtained from curcuminoid standards. All measurements were performed in triplicate and averages were calculated."

3.3.2 DPPH analysis

10 mg of solute are added in 10 mL flask, and diluted with dymethyl sulfoxide assisted by ultrasound. The method used has been the same that described by T. Perko et al. [3].

"DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured using the UV-VIS spectrophotometric method [23]. […] To a dark flask containing 77 µL of extract solution, 3 mL of DPPH solution was added. The mixed solutions were kept in the dark for 15

min at room temperature and the absorbance was measured using UV-VIS spectrophotometer (Varian, USA) at 515 nm. Radical-scavenging activities of samples were calculated using:

$$\%inhibition = \frac{A_B - A_A}{A_B} \cdot 100 \qquad 3$$

Where A_B is absorbance of blank sample (t = 0 min) and A_A is absorbance of extract solution (t = 15 min). All measurements were performed in triplicate and averages were calculated."

Samples get frozen in the fridge between the preparation of solutions and analysis due the melting point of DMSO.

4. Results

4.1 Extract yield

The extract yield has been calculated according the equation 4.

$$E = \frac{m_e - m_f}{m_c} \times 100 \qquad 4$$

Being E the extract yield, m_e the mass of the flask and the extract after the evaporation process, m_f the mass of the empty flask and m_c the mass off curcuma filled in the autoclave.

The amount of extract obtained in different times and temperatures, obey the figure 17.

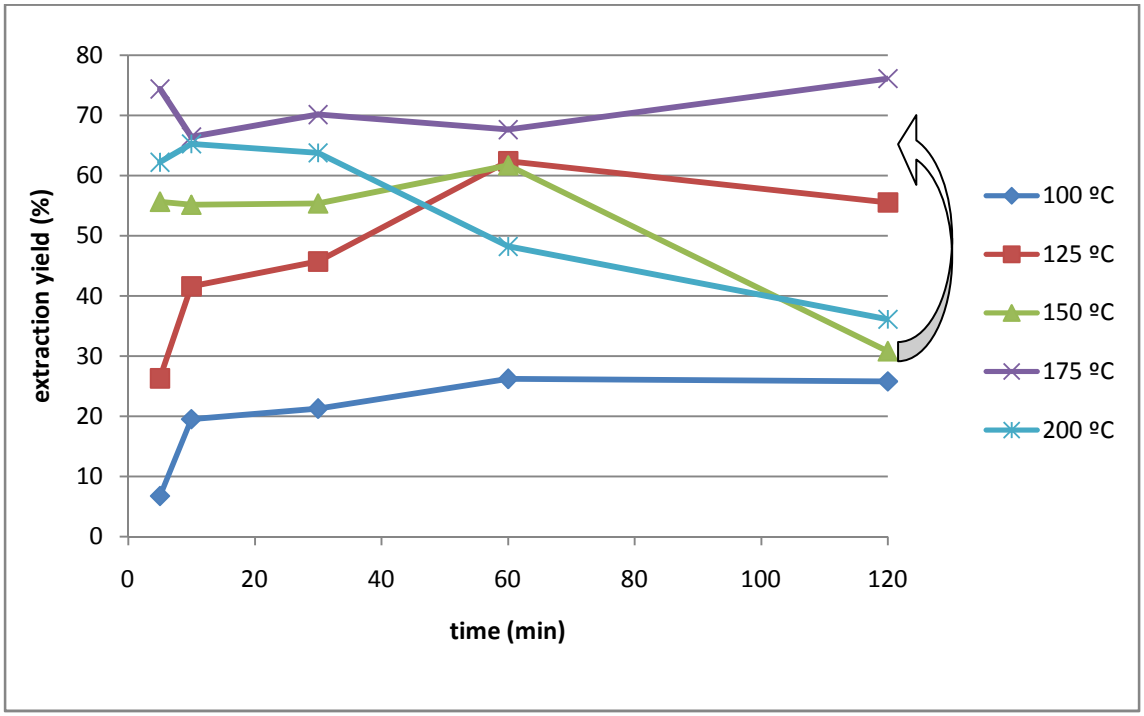


figure 17: plot of extraction yield at different times and temperatures. Values of experiments done twice figure as average.

Extraction yield increases with increasing temperature until 175ºC. The yield increases with time at lowers temperatures, being practically constant at 175ºC, and decreasing with increasing time at 200ºC.

The extract yield at 175ºC has three local maximums and two local minimums in a five points function. The range between global maximum and minimum is less than 10%, so the yield has been considered constant at this temperature, being 71%.

The extract yield at 150ºC, 120 minutes is lower (31%) than the expected one, being this one outside of the results after 120 minutes between temperatures at 125ºC (56%) and 175ºC (76%). This lack of coherence may be caused by the existence of starch in the sample, that hampers the filtration, bringing about the need of various filters instead one, even though a previous centrifugation. The analysis was also hindered due the low solubility of starch in water or methanol, being used dymethyl sulfoxide as solvent to avoid this problem.

4.2 Curcuminoids in extract

The amount of curcuminoids in each simple is shown in figure 18.

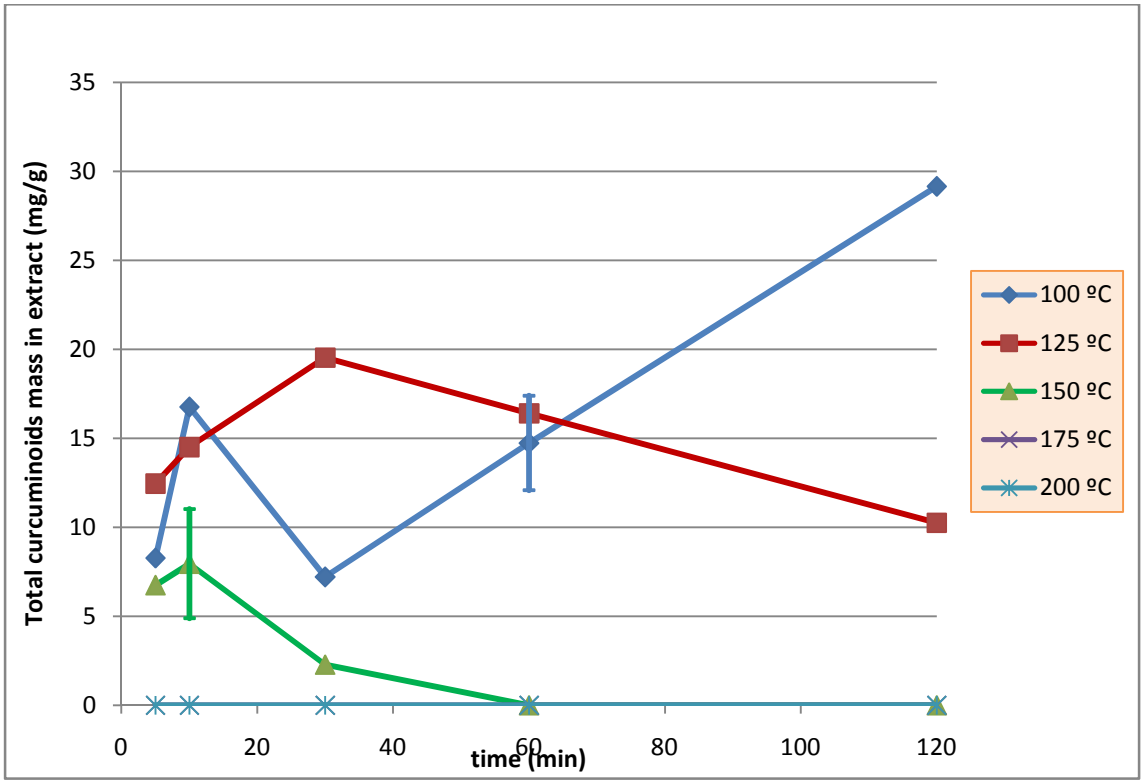


figure 18: total curcuminuids extracted for each time and temperature and time.

It can be seen that the highest quantity of curcuminoids is obtained at the lowest temperatures, 100 and 125ºC, being zero at temperatures higher than 150ºC. A reduction of the mass of curcuminoids can be seen at 125ºC and 150ºC while time increase, so curcuminuids are degraded at temperatures higher than 125ºC.

As has been shown before, in methanol solution, curcuminoids are almost in keto-enol isomer, being the amount diketo almost null. These substances are always in equilibrium, so during the separation process that the HPLC entail, they are together, taking place instantly the tautomeric reaction in other case. The relation between the mobile phases is monotone, so differentiable peaks for each isomer are not expected.

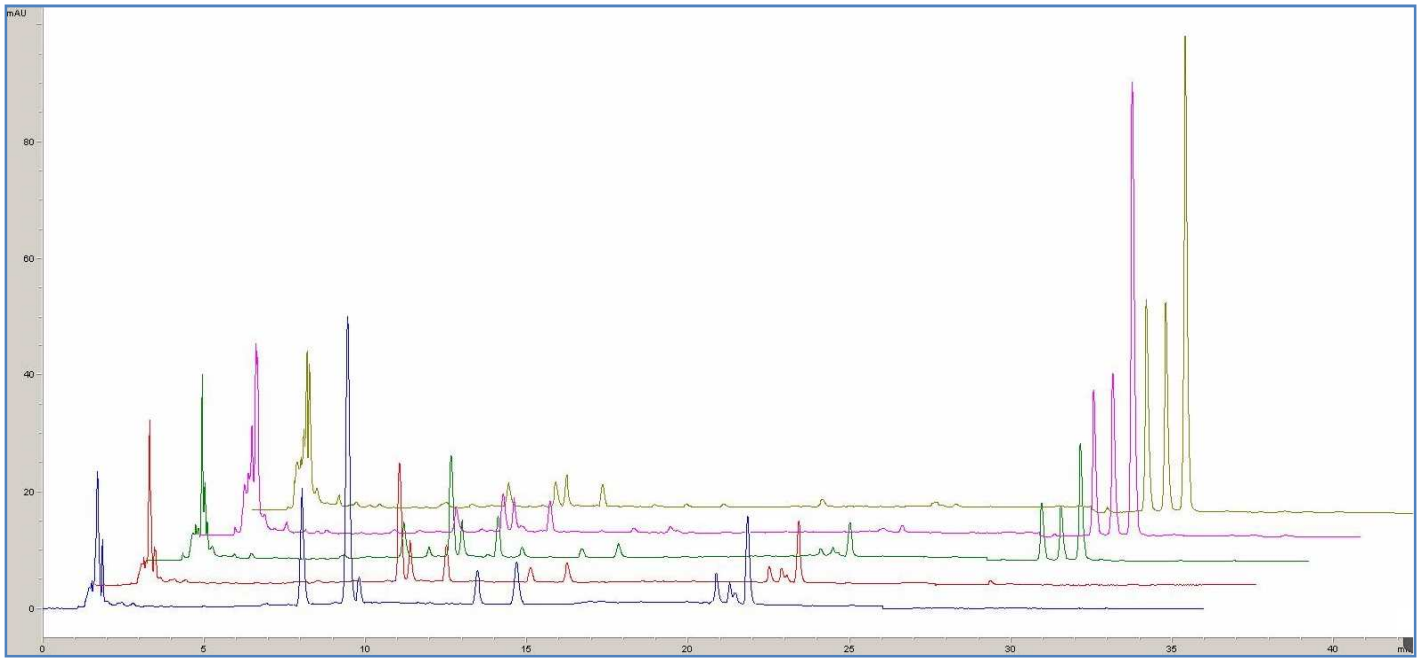


figure 19: from the top to the bottom, results of HPLC for extracions at rising temperatures (100, 125, 150, 175 and 200ºC) after 30 minutes. Curcuminuids are the peaks at 26 to 28 min.

figure 19 shows the behavior of samples obtained at the same time of extraction (30 min) at different temperatures. Curcuminoids are compounds whose outflow takes place after 25 minutes. By increasing the temperature of extraction the decomposition of curcuminoids occurs, and new substances are formed, as described before: vanillin, p-coumaric acid, ferulic acid and 4-Hydroxy-benzaldehyde, at 21-22, 13-15 and 7-9 minutes.

4.3 Antioxidant capability

Results of antioxidant capability of extract are given in figure 20.

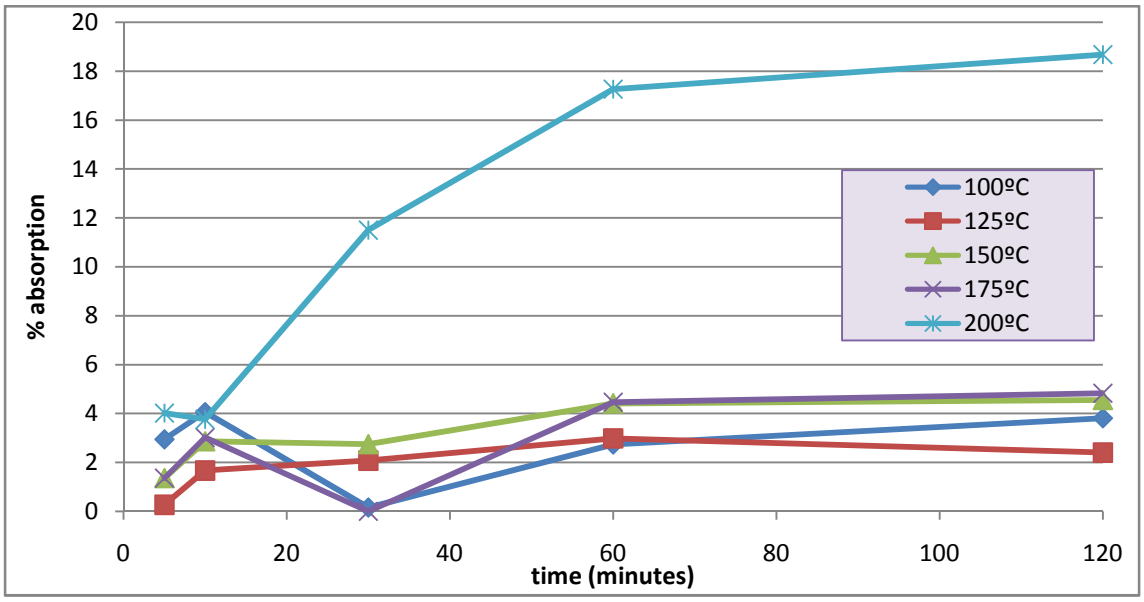


figure 20: Antioxidant capability, % inhibition for all temperatures and times.

The antioxidant capability is shown as % inhibition, being determined by DPPH method, where the free radicals of DPPH have to be scavenged by the antioxidants acting in the oxidative process.

The figure 20 shows a low level of antioxidant capability for all samples but the ones obtained at 200ºC. The antioxidant activity of extracts obtained at 200ºC increases with time of extraction until 18% after 120 minutes of extraction.

Extractions at 100ºC, 30 min and 175ºC, 30 min have an antioxidant activity measurement zero value, value that is not expected. These results have to be taken as mistakes.

Color and bright

A quality measurement has been taken. Figure 21 shows the color of each sample.

Time/temp	100	125	150	175	200
005					
010	XXXXXXXX				
030					
060					
120					

figure 21: color for each sample.

The yellow color is brighter at lower temperatures, specifically at 125ºC and short times, this agrees with the curcuminoids analysis.

More particular explanations will be done for each temperature. A further qualitative description is also given.

4.5 Results of extractions at 100ºC

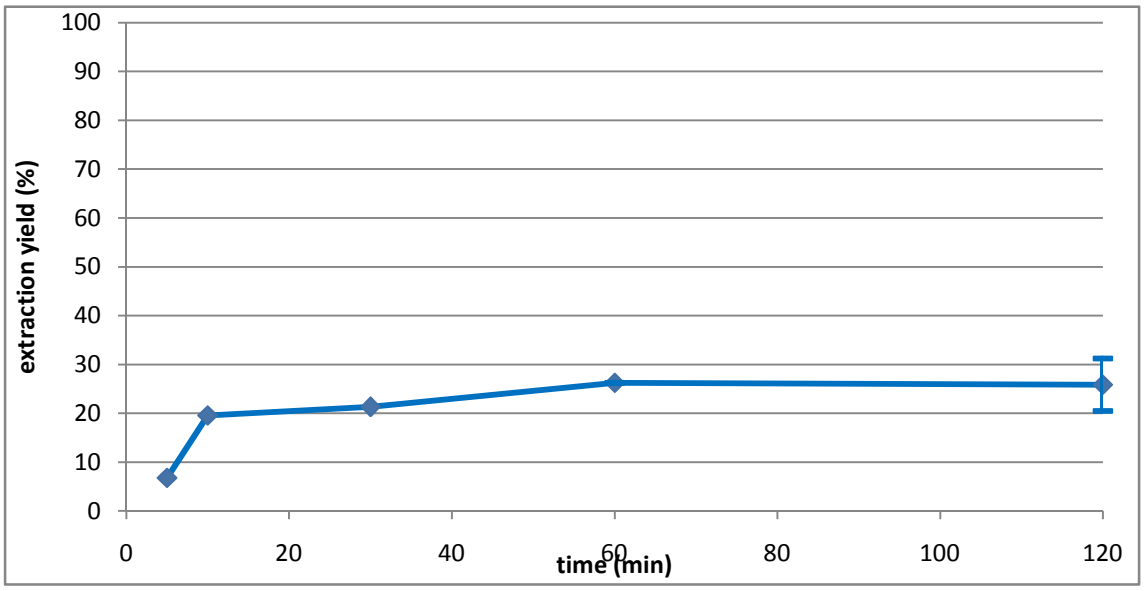


figure 22: Extraction yield at 100ºC, single values and averages ( ), errors ( ).

At the lowest temperature, 100ºC, the mixture obtained in the extractor is a bright yellow solution, good smell, and can be filtrated without problems, obtaining a dry porous solid and a yellow solution.

At this temperature, the maximum yield of extraction is obtained in short times. After one hour of extraction the yield doesn't increase any more. The extraction yield is low, never higher than 30%.

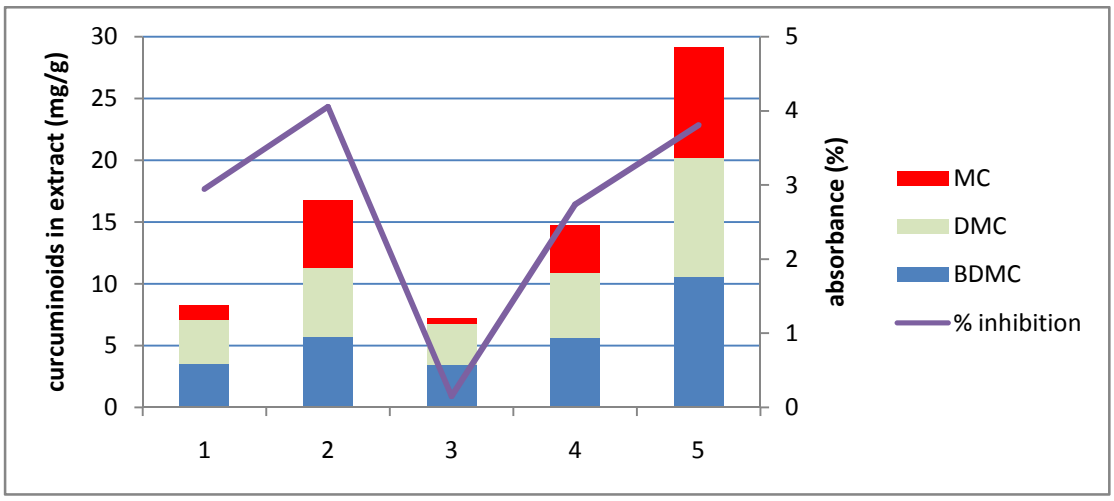


figure 23: HPLC & DPPH results for 100ºC extractions at 5 min (1), 10 min (2) 30 min (3) 60 min (4) and 120 min (5)

The amount of curcuminoids increases with time of extraction, until close to 30 mg/g of extract (the amount of curcuminoids in turmeric is between 3 and 5%). Bis-demethoxy curcumin is the most abundant in al extracts. The absorbance maximum value of absorbance is 4% (10 minutes and 120 minutes). There is a direct relation between curcuminoids content and antioxidant capability.

4.6 Results of extractions at 125ºC

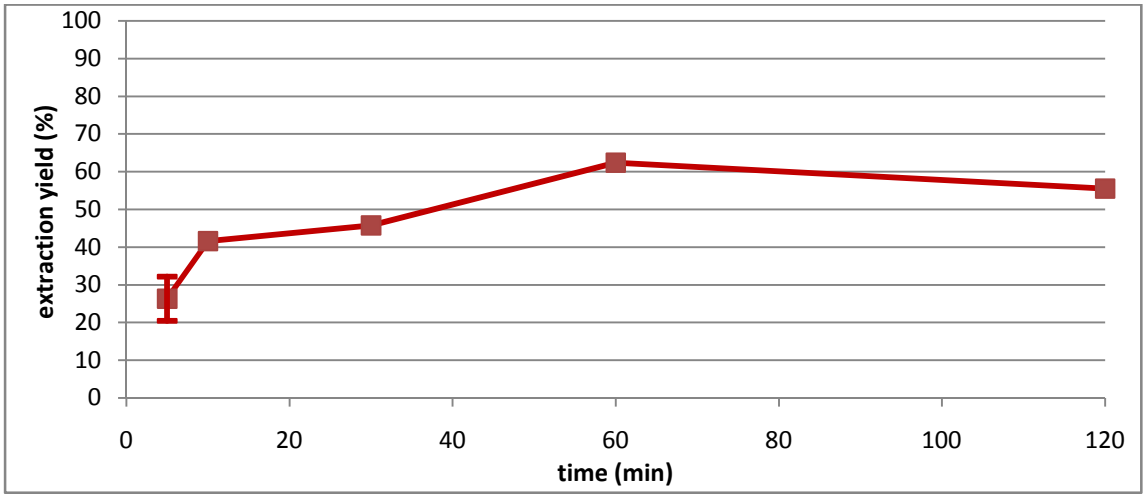


figure 24: Extraction yield at 125ºC, single values and averages ( ), errors ( ).

At 125ºC the extract are more or less the same in appearance and smell as 100ºC extract. At 125ºC and 30 min there were problems with the filtration; the flow in the filter was close to zero and was zero after few seconds. To avoid this, the solution is centrifugated previously (two minutes 11000 rpm) to remove the colloid that obstructs the filter. After centrifugation the flow decreases with time, but doesn't stop before the solid is dry.

At 125ºC the extraction yield increases with time, being the maximum around 60% and almost constant after one hour. Five minutes at this temperature is enough to obtain the same extract as the optimums at 100ºC, after 60 minutes of extraction.

The content of curcuminuids is the highest at 30 minutes of extraction and afterwards it decreases with time what shows that at this temperature decompositions start to take place. The composition of the sample after 30 minutes is consistent with the fact that curcumin is the most abundant component, but it loses the methoxy radical to become demethoxy-curcumin or bis-demethoxy-curcumin; with time, all of them are decomposed to easier compounds.

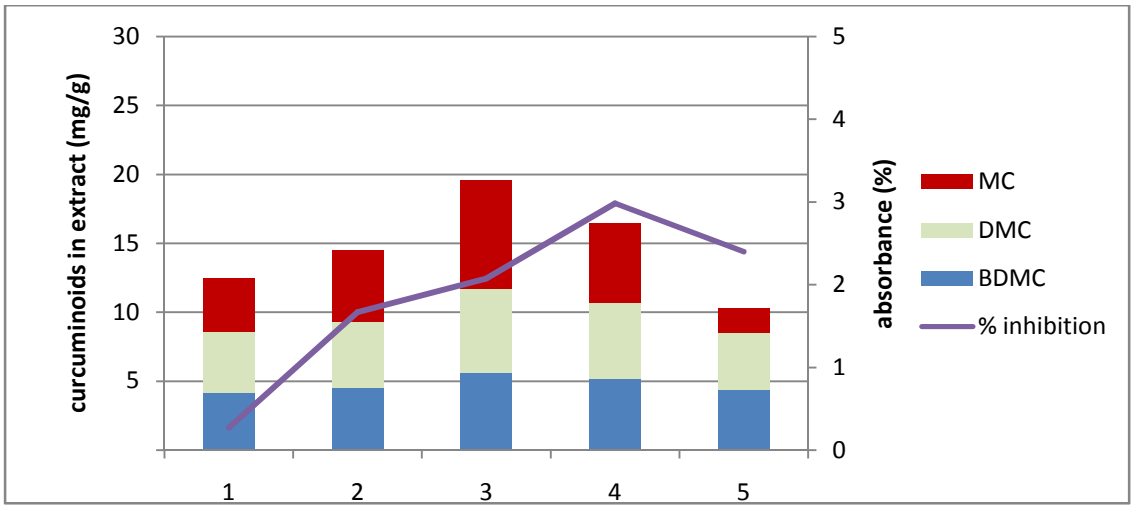


figure 25: HPLC & DPPH results for 125ºC extractions at 5 min (1), 10 min (2) 30 min (3) 60 min (4) and 120 min (5)

It's also observable that the amount of demethoxy curcumin and bi-demethoxy curcumin remain constant during the first 30 minutes, and just the amount of curcumin increases. It can be understandable that the mass transfer of the last one is slower than the DMC and BDMC. The antioxidant activity rises until 3% after one hour of extraction.

4.5 results of extraction at 150ºC

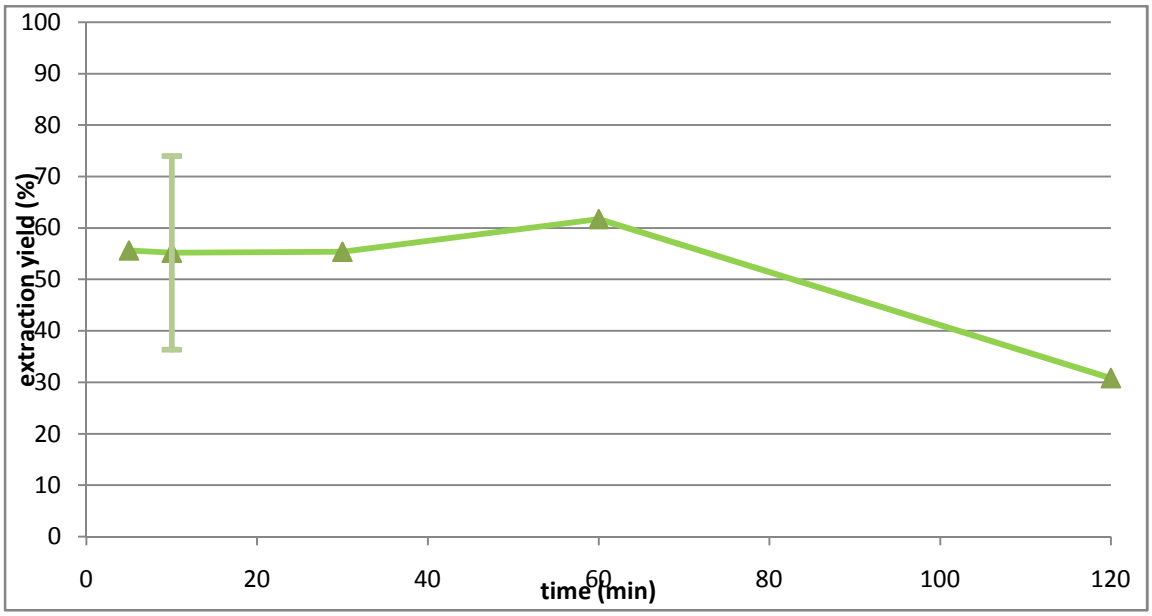


figure 26:Extraction yield at 150ºC, single values and averages ( ), errors ( ).

At 150ºC there are again problems with filtration. To avoid this, the sample is centrifugated for 6 minutes at 11000 spins per minute. Anyway, the filtration requires a lot of time (20-30 minutes for 60 mL of solution), and news filters (up to 6 in the worst situation, what causes high losses of extract. The appearance of the mixture is yellow too, but more brown or red than at 125ºC. The smell is still good.

The yield of extraction is approximately constant in time period from 5 to 60 minutes (60%) and afterwards it decreases to approximately 30% after 120 minutes of extraction. The big difference between errors of samples at10 minutes is due the difficulties in the filtration.

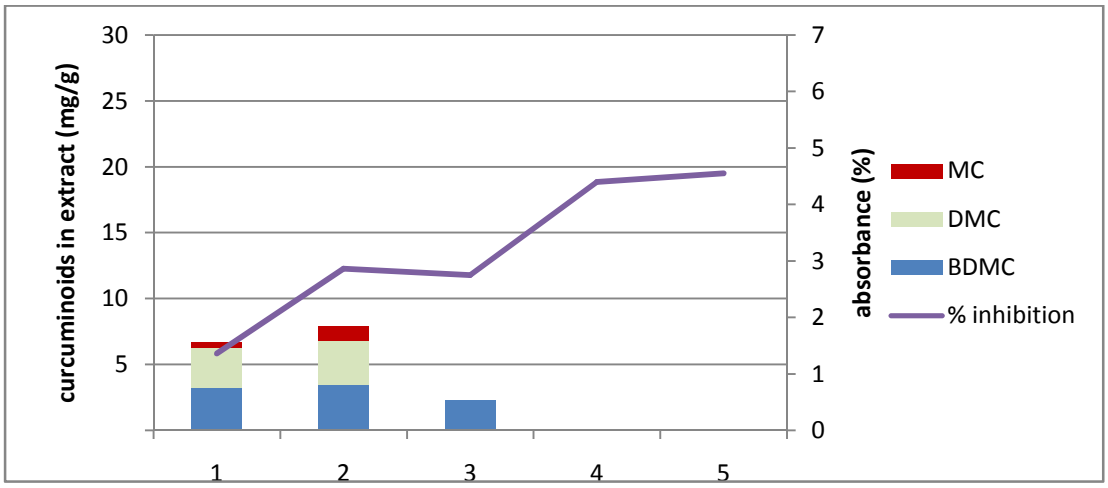
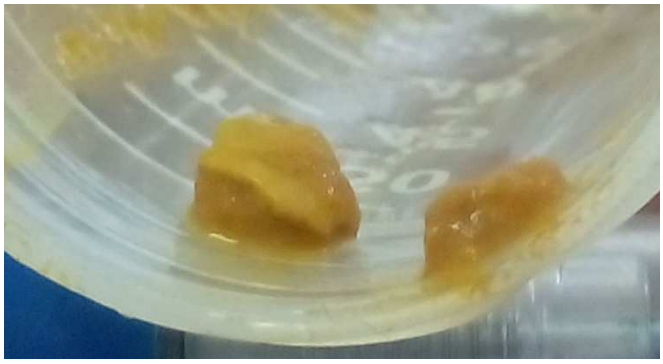


figure 27: HPLC & DPPH results for 150ºC extractions at 5 min (1), 10 min (2) 30 min (3) 60 min (4) and 120 min (5)

The amount of curcuminoids in extact is smaller than at lower temperatures, reaching zero values after 60 min. The amount of curcumin is almost zero or zero in all the samples, and there is just bis-demethoxy curcumin after 30 minutes of extraction, so the decomposition of the methoxy groups is also expected at 150ºC. On the other hand, the antioxidant capability rises with time, while curcuminoids amount in extract decreases.



After the centrifugation, the solid is stratified, with a big amount of brown substance and a thin layer of brighter yellow material.

figure 28: stratification of the solid residue after centrifugation

4.5 results of extraction at 175ºC

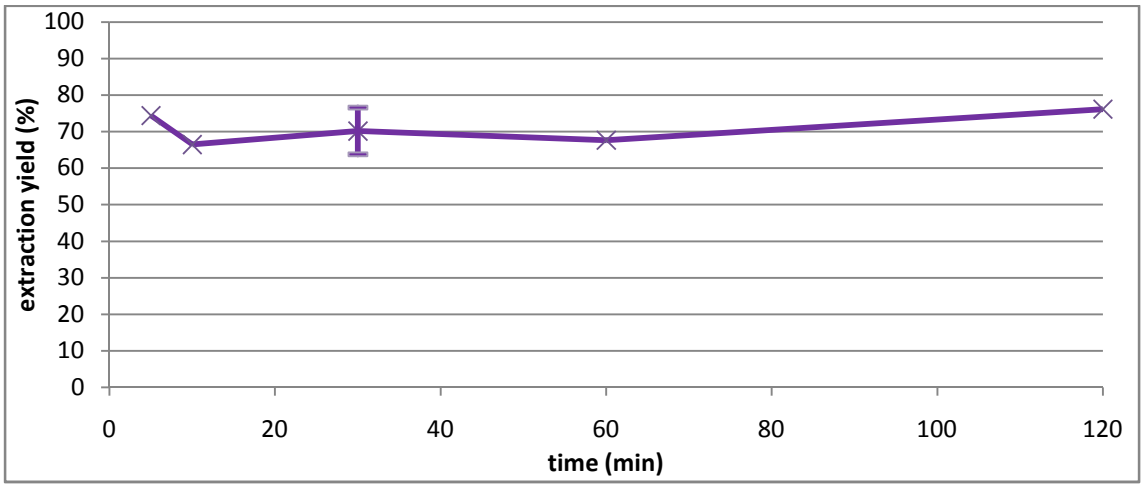


figure 29:Extraction yield at 175ºC, single values and averages ( ), errors ( ).

At 175ºC and short times of extraction until 10 minutes, the properties of products are close to the ones obtained at 150ºC and longer times; a yellow to brown mixture is obtained that is bad filterable also after centrifugation. If the time is 30 minutes or higher, the mixture starts to be brown, and it's easy filterable, so the sample is not centrifugated. The smell is lightly unpleasant, and softer than of the ones samples obtained at lower temperatures.

Extraction yield at 175ºC is almost constant with time (around 70%), and is also the biggest.

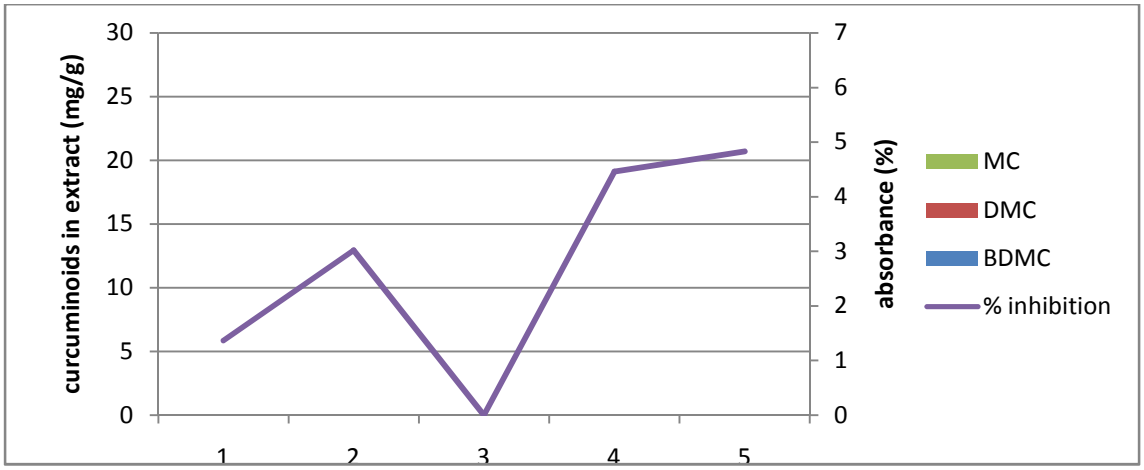


figure 30: HPLC & DPPH results for 175ºC extractions at 5 min (1), 10 min (2) 30 min (3) 60 min (4) and 120 min (5)

The amount of curcuminoids is zero regardless of extraction time, what indicates that total decomposition of curcuminoids has taken place even in the shortest times. Antioxidant activity

reaches low values of 5% after 1 or 2 hours. The antioxidant activity result is zero if time is 30 min, this unexpected result is probably an experimental error.

figure 31: comparation of color of samples at 175ºC, 10 and 60 minutes

The color of the solid waste after filtration is absolutely different after 10 to 60 minutes of extraction due to the Maillard reactions that occur at these temperatures with organic compounds. The waste obtained after 10 minutes 175 º C samples is yellow, but doesn't have the bright as samples at lower temperatures had. The Maillard's reaction has started after 10 minutes, but his effects are not so visual as after 60.

4.9 Results of extractions at 200ºC

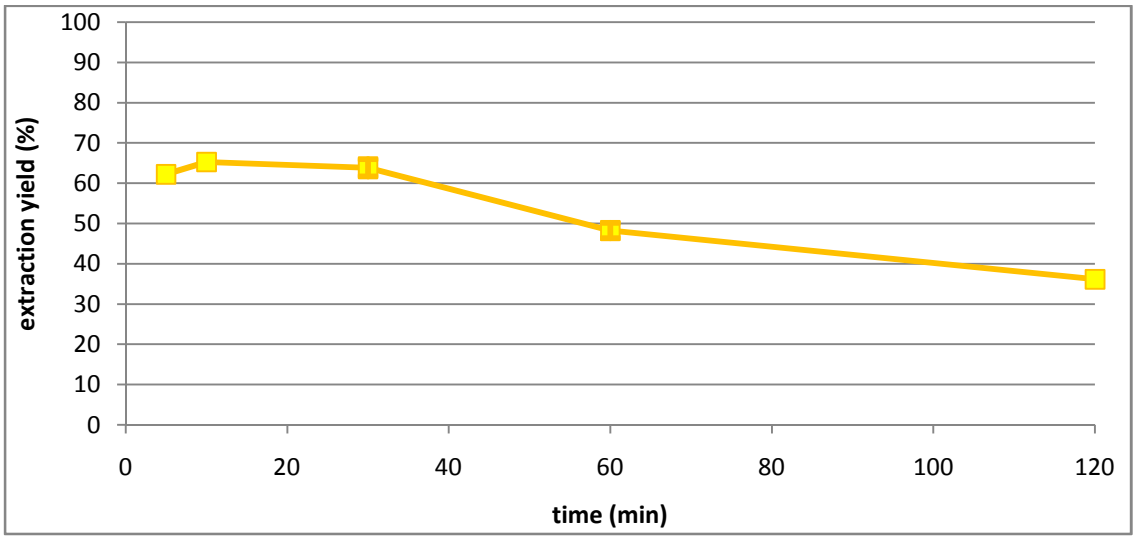


figure 32:Extraction yield at 200ºC, single values and averages ( ), errors ( ).

The properties of the product obtained at 200ºC are close to the ones at 175ºC, The color strength obtained after the filtration is lower than of the product obtained at 175ºC. The smell is unpleasant too, more deep than of the previous one, but still softer than of the samples obtained at 150ºC and lower temperatures, and has a toasted connotation. The extract after evaporation is not a solid, but an oily substance.

The extraction yield is high, not so high as at 175ºC, but close to 70%, at short times of extraction. Extraction yield decreases after 30 and reaches being 40% after two hours.

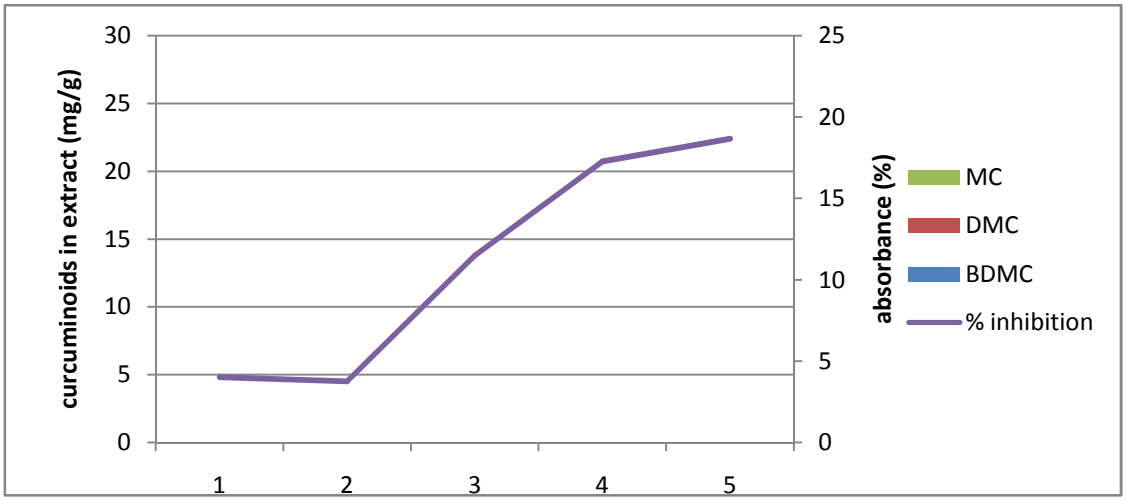


figure 33: HPLC & DPPH results for 200ºC extractions at 5 min (1), 10 min (2) 30 min (3) 60 min (4) and 120 min (5)

Similar as at 175ºC, all the curcuminoids have been degraded to simpler compounds, while the antioxidant activity increases with time. At this temperature the antioxidant activity increases until 18%, what is more than three times higher as at lower temperatures. This value is still lower than the values obtained with other extraction procedures (24% for the best supercritical CO2 extraction, 35% for traditional extraction with ethanol) [3].

5. Conclusions:

175ºC is high enough temperature to degrade the total amount of curcuminoids already in 5 minutes. At 150ºC the same result is obtained after 30 minutes of extraction.

The degradation of curcuminoids can be considered zero at 100ºC in pressurized water.

The best conditions for curcuma extraction are 125ºC and 60 min, in order to obtain the maximum amounts of curcuminoids; or 125ºC, 30 min to have me most concentrated extract. These maximums are 10.2mg/g curcuma (125º, 60 min) and 19.53mg/g extract (125, 30 min). The best values of antioxidant activity are obtained at the highest temperature, after 1 and 2 hours, being 18%. All these values are lower than those obtained by the traditional extraction with ethanol. This fact reveals this method as not the best one for this process.

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7. Appendix

Table 5: extraction yield of all samples

sample	curcuma mass	empty flask	extract and flask	extract	extract yield
100ºC;5min	1.5006	167.5599	167.6616	0.1017	6.78
100ºC;10min	1.5038	181.7532	182.047	0.2938	19.54
100ºC;30min	1.5002	167.3875	167.7069	0.3194	21.29
100ºC;60min	1.5016	181.7532	182.1467	0.3935	26.21
100ºC;60min	1.4999	---	---	0.3937	26.25
100ºC;120min	1.5007	167.3871	167.855	0.4679	31.18
100ºC;120min	1.5007	---	---	0.3072	20.47
125ºC;5min	1.5007	---	---	0.3072	20.47
125ºC;5min	1.5001	---	---	0.4828	32.18
125ºC;10min	1.5002	167.2853	167.91	0.6247	41.64
125ºC;30min	1.5009	167.3895	168.0762	0.6867	45.75
125ºC;60min	1.5044	167.2853	168.224	0.9387	62.40
125ºC;120min	1.4997	172.7764	173.6096	0.8332	55.56
150ºC;5min	1.499	167.3854	168.2195	0.8341	55.64
150ºC;10min	1.4991	167.3854	167.9308	0.5454	36.38
150ºC;10min	1.5006	225.56	226.67	1.11	73.97
150ºC;30min	1.5013	167.3897	168.2216	0.8319	55.41
150ºC;60min	1.5013	167.3897	168.3164	0.9267	61.73
150ºC;120min	1.5003	173.5291	173.9912	0.4621	30.80
175ºC;5min	1.5	167.3858	168.5022	1.1164	74.43
175ºC;10min	1.4994	167.3886	168.3854	0.9968	66.48
175ºC;30min	1.4992	167.3886	168.3443	0.9557	63.75
175ºC;30min	1.5001	167.389	168.5378	1.1488	76.58
175ºC;30min	1.5001	167.389	168.5378	1.1488	76.58
175ºC;60min	1.4997	172.6738	173.6885	1.0147	67.66
175ºC;120min	1.5007	167.3862	168.5289	1.1427	76.14
200ºC;5min	1.4982	167.3886	168.321	0.9324	62.23
200ºC;10min	1.4991	167.3886	168.3675	0.9789	65.30
200ºC;30min	1.5003	167.3871	168.3126	0.9255	61.69
200ºC;30min	1.5	167.3882	168.3776	0.9894	65.96
200ºC;60min	1.5024	169.3673	170.0636	0.6963	46.35
200ºC;120min	1.5002	167.3886	168.1408	0.7522	50.14
200ºC;120min	1.5034	167.3896	167.9331	0.5435	36.15

Table 6: HPLC results: mass of curcuminuids

sample	HPL mass	BDMC area	DMC area	C area	BDMC mg/ g ext.	BMC mg/g ext.	C mg/ g ext.	total mg/g ext.
100ºC;5min	20.44	114.05	115.45	243.90	3.53	3.54	1.19	8.27
100ºC;10min	20.13	275.30	266.90	615.35	5.68	5.64	5.43	16.75
100ºC;30min	20.09	107.95	96.85	180.20	3.45	3.29	0.47	7.20
100ºC;60min	19.86	321.30	297.15	580.90	6.30	6.06	5.04	17.39
100ºC;60min	17.88	216.30	192.05	365.30	4.90	4.60	2.58	12.08
100ºC;120min	---	---	---	---	---	---	---	---
100ºC;120min	21.35	642.20	554.75	923.55	10.58	9.62	8.95	29.15
125ºC;5min	23.43	164.20	174.90	480.15	4.20	4.36	3.89	12.46
125ºC;5min	---	---	---	---	---	---	---	---
125ºC;10min	26.1	188.00	206.50	593.70	4.52	4.80	5.19	14.51
125ºC;30min	22.07	267.50	300.60	827.35	5.58	6.10	7.85	19.54
125ºC;60min	24.03	237.10	257.25	641.20	5.17	5.50	5.73	16.41
125ºC;120min	22.86	174.10	161.40	291.80	4.33	4.18	1.74	10.25
150ºC;5min	21.44	92.65	79.95	177.95	3.25	3.05	0.44	6.74
150ºC;10min	10.8	35.75	32.55	65.75	2.49	2.40	0.00	4.88
150ºC;10min	23.84	185.75	169.10	335.75	4.49	4.28	2.24	11.01
150ºC;30min	22.12	20.50	0.00	0.00	2.28	0.00	0.00	2.28
150ºC;60min	21.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150ºC;120min	22.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;5min	21.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;10min	12.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;30min	---	---	---	---	---	---	---	---
175ºC;30min	26.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;30min	23.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;60min	26.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
175ºC;120min	28.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;5min	20.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;10min	23.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;30min	27.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;30min	26.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;60min	22.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;120min	24.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200ºC;120min	23.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 7: DPPH results. antioxidant capability.

	anti-oxidative mass	valor	% inhibición
100ºC;5min	10.1	0.8335	2.95
100ºC;10min	9.31	0.824	4.05
100ºC;30min	11.25	0.8575	0.15
100ºC;60min	9.98	0.8339	2.90
100ºC;60min	10.44	0.8367	2.57
100ºC;120min	---	---	---
100ºC;120min	10.88	0.8261	3.81
125ºC;5min	9.91	0.8565	0.27
125ºC;5min	---	---	---
125ºC;10min	11.56	0.8445	1.67
125ºC;30min	10.17	0.841	2.07
125ºC;60min	11.29	0.8332	2.98
125ºC;120min	11.4	0.8382	2.40
150ºC;5min	9.59	0.8471	1.36
150ºC;10min	10.02	0.8448	1.63
150ºC;10min	10.48	0.8236	4.10
150ºC;30min	12.13	0.8352	2.75
150ºC;60min	10.73	0.821	4.40
150ºC;120min	13.58	0.8197	4.55
175ºC;5min	11.82	0.8471	1.36
175ºC;10min	9.09	0.8329	3.02
175ºC;30min	---	---	---
175ºC;30min	10.1	0.8771	-2.13
175ºC;30min	---	---	---
175ºC;60min	10.69	0.8205	4.46
175ºC;120min	10.16	0.8173	4.83
200ºC;5min	9.78	0.8243	4.02
200ºC;10min	10.23	0.8265	3.76
200ºC;30min	9.76	0.7542	12.18
200ºC;30min	10.11	0.7659	10.82
200ºC;60min	10.1	0.7105	17.27
200ºC;120min	9.88	0.7237	15.73
200ºC;120min	12.78	0.6731	21.62