The Fortification's Feasibility of the Butter by the Polyphenols Present in the Olive Waste

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Abstract— *In spite of their polluting nature, the olive mill wastewater is considered as a very rich source on natural antioxidants specially polyphenols.*

In this study, the polyphenols were extracted from the olive pomace and the olive mill wastewater in order to promote them as a natural antioxidants and to compare its with a synthetic antioxidant β-carotene. Those polyphenolswill be added to the butter. To quickly assess the effect of polyphenols on the stability of the butter, we conducted an accelerated oxidation. Then the butter was placed under storage conditions in an oven (accelerated test at 60 °C) for 28 days. The evolution of the oxidation state was measured by the peroxide value and acidity. The results showed that the butter containing antioxidants have undergone oxidative damage less pronounced than that of the reference (no additives).

Keywords— predicting, survey & Research.

I. INTRODUCTION

In Morocco, the olive oil sector plays a very important role in socio-economic terms. It actively contributes to the setting of rural populations, providing more than 15 million days of work [1]. With a national production of 700 000 tons of olive oil, Morocco occupies the 6th place after Spain, Italy, Tunisia, Turkey, and Greece [2].The olive oil industry produces in addition to oil which is considered as the main product, there is a large amounts of olive mill wastewater (OMW) and solid waste (olive pomace, leaves and pruning). The annual quantities of vegetable represent a volume of 685 000 tones and the pomace were estimated at 255,000 tones [2].

The toxicity of the olive mill waste water is attributed to the presence of free fatty acids and the long chain recalcitrant compounds difficult to degrade as phenolic compounds, in high concentrations (4-15gL-1), which are responsible of phytotoxic effects and antimicrobial [3]. Despite their pollutant profiles, olive mill waste water is considered as a rich source of natural antioxidants specially the polyphenols. Moreover, the polyphenols have antimicrobial properties, hypolipidemic, cholesterol lowering and anti-cancer [4].

Therefore, the food industry is developed the use of natural antioxidants. At the present, the polyphenols capture the interest of researchers to find new raw materials abundantly available [5]. The recover the polyphenols from the olive waste present in addition to consumer safety, two advantages: firstly, solving a major environmental problem; and secondly, use the polyphenols in future food, cosmetics or pharmacology applications. The present work aims to extract the polyphenols in olive residues and evaluate their antioxidant powers and to verify the stability of the butter to enhance those polyphenols.

II. PROCEDURE

2.1 Olive waste

The olive mill wastewater and pomace olive are recovered of a modern industrial unit olive crushing by centrifugation at three stages, located in the region of Fez.

2.2 Preparation of phenolic extract

2.2.1 Preparation of methanol extract

10g of pomace olive (or 10mL of olive mill wastewater) was extracted by shaking at 150 rpm for 24 h with 100 ml methanol (80%) and filtered through filter paper No 1. The filtrate designated as methanol extract. The experiment was performed at triplicate [6].

2.2.2 Estimation of the phenolic content

The total phenolic content was estimated using Folin- Ciocalteu (FC) assay which is widely used in routine analysis [7]. Briefly, 0.16 ml (three replicates) of the extracts diluted in distilled water was mixed with 0.16ml of FolinCiocalteu reagent. After 5min, 1.6 ml of 7% sodium carbonate were added. The absorbance of the resulting blue colored solution was measured at 750 nm after 90 min with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of point from 0 to 0.5mg/ml of Gallic acid in methanol. The total phenolic content was expressed as Gallic acid (GAE) in mg/100g of extract.

2.2.3 Evaluation of antioxidant activity

Antioxidant activity was evaluated by Scavenging Radical activity using 2,2-Diphenyl-2-picryl hydrazyl radical method (DPPH) was adapted from protocol of Hanato [8] with some modifications. Each sample stock solution (0.2mg/ml) was diluted to final concentrations of 0.01, 0.02, 0.03, 0.04 mg/ml, in methanol, and 0.5 Mm DPPH methanol solutions. Sample solution of different concentrations was allowed to stand at room temperature in the dark. After 30min, the decrease in absorbance at 517 nm was measured. The antiradical efficiency is calculated as follows:

$$EA = \frac{1}{IC50}$$

Along With: IC50 is the concentration of extract required for obtaining 50% of the reduced form of the DPPH radical.

2.3 Preparation of the butter

2.3.1 Butter analysis used

A series of test tubes were placed in a temperature-controlled oven at 60 °C for 28 days to study the stability of the butter concerned in storage conditions. The photo-oxidative activity was achieved by the introduction of a 40 watt lamp in the oven. Tree concentrations of phenolic compounds (4, 8 and 12 mg), and the control of β-carotene were respectively added to the analyzed butters and the butter plus a series of control in order to compare and assess their effects antioxidants.

Throughout the storage Periodic removal of a 1 cm at a rate of once every 7 days three samples of each series were served to further deterioration of the oils as a measure of oxidation.

2.3.2 Measuring the peroxide

Five gram of butter are dissolved in a mixture of 25 mL of acetic acid / chloroform acid (3/2 v: v) and 1 mL of a saturated solution of potassium iodide. After 5 min reacting, in the dark, 75 mL of distilled water are added and the liberated iodine is titrated with a sodium thisulfate solution 0.1 N in the presence of starch paste. A control test (without fat) is performed in the same conditions.

2.3.3 Determination of acidity

Acidity is the percentage of free fatty acids in a fatty substance.

The determination of acidity of oils was carried out according to the AFNOR NF T60-204whose is a test sample which bring into the solution in a solvent mixture (ethanol / diethyl ether) and as the fatty acids present by means of a potassium hydroxide solution in the presence of phenolphthalein as a color indicator.

III. RESULTS AND DISCUSSIONS

3.1 Antioxidant activity

The antioxidant activity of the phenolic extracts of the olive pomace, the olive mill wastewater and the standard antioxidant (vitamin C, BHT) towards the DPPH radical is evaluated by the trapping of the free radical DPPH test.

The results of the phenolic antioxidant extracts EA of the olive pomace and the olive mill wastewater (Table I) show that the percent inhibition of these extracts are superior to standard inhibition percentage. IC50 is the concentration of sample necessary to obtain 50% of the reduced form of the DPPH radical. Indeed, the transformation of the free radical DPPH.DPPH-H antioxidant is covered by the reaction:

$$DPPH + AH \rightarrow DPPH\text{-}H + A.$$

Where AH is a compound capable of donating H in DPPH radical?

TABLE 1
IC50 AND EA OF EXTRACTS

	Vitamin C	BHT	Olive Pomace	Olive mill wastewater
IC50 (mg/mL)	0,005	0,006	0,15	0,09
EA	200	166,66	6,66	11,11

According to the results recorded, extracts of olive pomace and olive mill wastewater feature a moderate antioxidant power, their respective IC50 are 0.15 mg/mL and 0.09 mg/mL but relatively lower than that of ascorbic acid with a value of about 0,005 mg/mL. It has been shown that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins reduce DPPH and discolored due to their ability to transfer hydrogen [9].

3.2 Measuring the peroxide

For the marketing of the butter, its lifespan is essential to determine a time gap between production and consumption [10]. To protect consumers, the legislation requires several parameters that describe the butter oxidation state. Indeed, the peroxide value is one of these parameters, the index increases gradually with the primary oxidation of butter until the rate of degradation of the hydroperoxides formed exceeds the formation of new hydroperoxides [11-12]. For the butter, the European standard sets the maximum value of this index to 10 MeqO2/kg of oil [13].

The butter without additives (control) has the highest peroxide value among others. In fact, the peroxide index was 2.994 MeqO2/kg before being subjected to the oven temperature (60 °C) and after 28 days of storage, it reaches 43 MeqO2/kg. A very marked increase (14 times) its initial peroxide value can be ascertained at the end of storage. The evolution of this index butters containing antioxidants follows a relatively less pronounced rate than the control. This shows that the enrichment of vegetable butter by polyphenols of olive mill wastewater (figure I) and of pomace (figure II) appears to improve the oxidative stability of the butter; the same explanation was advanced by other studies confirmed that the polyphenols increase the stability of the oils [14].

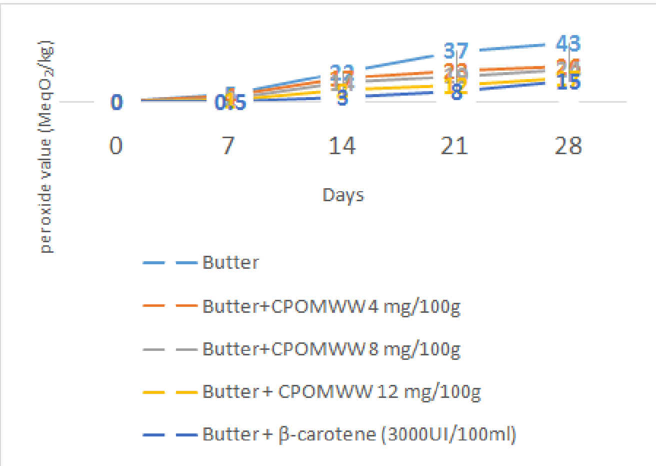

FIG. 1 EVOLUTION OF THE PEROXIDE VALUE: BUTTER COMPLEMENTED BY OLIVE MILL WASTEWATER PHENOLIC COMPOUNDS (CPOMWW)

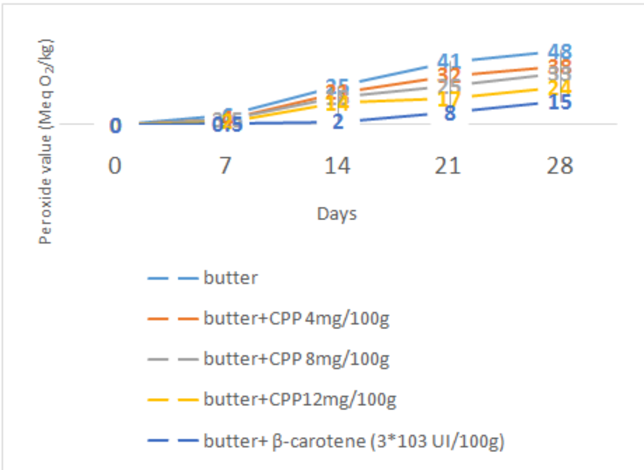

FIG. 2 EVOLUTION OF THE PEROXIDE VALUE: BUTTER COMPLEMENTED BY POMACE PHENOLIC COMPOUNDS (CPP)

However, we record the evolution of the butter peroxide value complemented by pomace olive is hagher than the olive mill wastewater.

This could be probably because the olive mill wastewaters are richer in antioxidants than the olive pomace.

3.3 Determination of acidity

Free acidity is an important factor in assessing the quality of butter, and is widely used both as a conventional commercial classification criterion [15]. In addition, a factor provides information on the alterations in the oil by hydrolysis [15]. Indeed, in vegetable oils, natural fatty acids are essentially present as triglycerides 98-99% [16]. Hydrolysis of these releases fatty acids that allow the assay to have an idea about the state of degradation of the oil [15]. The results obtained (figure III and IV) showed little differences in highly significant between the beginning and the end of the experiment. The small changes in acidity during storage can be explained by triglyceride hydrolysis which is not sufficient to offset or increase the free fatty acid groups blocked by polymerization or volatilized during oxidation phase [17].

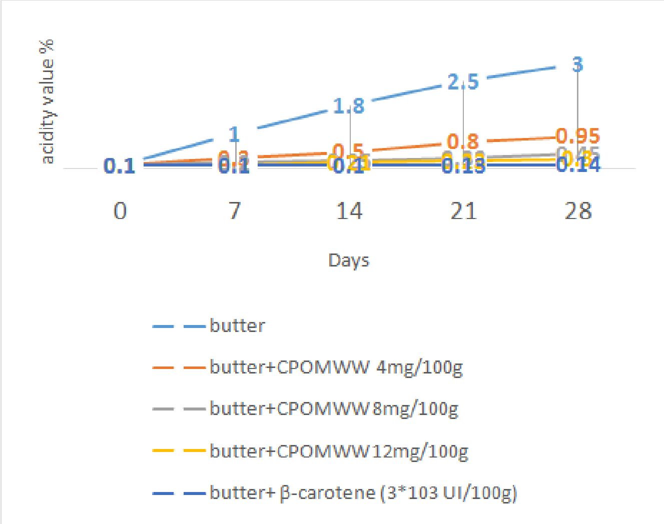

FIG. 3 EVOLUTION OF ACIDITY VALUE: BUTTER COMPLEMENTED BY OLIVE MILL WASTEWATER PHENOLIC COMPOUNDS (CPOMWW)

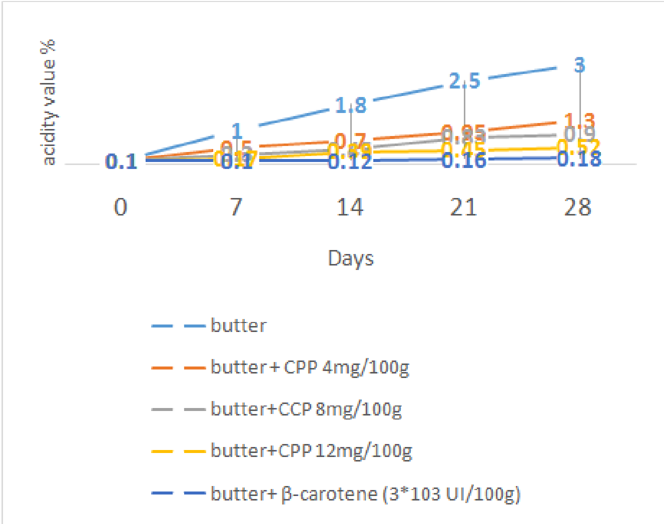

FIG. 4 EVOLUTION OF ACIDITY VALUE: BUTTER COMPLEMENTED BY POMACE PHENOLIC COMPOUNDS (CPP)

The acidity change is slight for all samples of the butter used. In any case, the acidity of butter examined does not vary significantly with the addition of polyphenols. Those results are consistent with those obtained by N. Denisse [18], who found that the acidity of turnsole oils, Nuts and Soybean that are added phenolic extracts remains constant for a storage period of 22 days at 60 °C. This trend has been noted by S. Fodil [19] when studying the effect of β-carotene and vitamin E on the oxidative stability of three types of virgin olive oil.

IV. CONCLUSIONS

The study of the antioxidant activity of the polyphenols of the mill wastewater and the pomace olive by trapping free radical DPPH method showed that the both polyphenols have a significant antioxidant activity and cheap. It is clear that this extracts polyphenols are effective antioxidants which have two important advantages. On the one hand, those polyphenols of the natural origin that are characterized by a high antioxidant capacity comparable to synthetic β-carotene; and can replace it in efficient use as food that does not involve the risks to the human health and it is more cheaply. Moreover, the environmental impact of vegetable has been reduced which would lead the countries of olive oil producers to take advantage of this raw material on an industrial scale as not economically expensive source, but rich in polyphenols. Indeed, during storage of 28 days in an oven (60 °C) butter has gained a better resistance to oxidation due to an addition of 12mg of polyphenols for 100g of butter.

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