UTILIZATION OF WINE INDUSTRIES' SPENT GRAIN IN BREAD ENRICHMENT

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By-products of the wine industry are rich in dietary fiber and proteins, and due to their high antioxidant capacity, grape seed flours provide additional health benefits. This additional effect was evaluated through the determination of total polyphenol content and total antioxidant activity via FRAP and DPPH assays in breads enriched with grape seed cake or grape seed flour. Mixing wheat flour with grape seed products significantly influenced the rheological properties of the dough and the quality parameters of the breads. In this context, defatted grape seed cake and conventional grape seed flour, in specific concentrations (5%, 7.5%, 10%, and 15%), were used to enrich commercial medium strong wheat flour and to develop new bread formulations. As expected, the enrichment led to a significant increase in the content of proteins, fibers, lipids, and ash, depending on the enrichment level, as well as a marked increase in the antioxidant capacity. The total deformation characteristics, plastic and elastic deformation of the breads crumb used as tools to determine the shelf life demonstrated the enriched breads freshness maintained within five to six days of storage which is entirely acceptable from the consumers' point of view.

MATERIALS AND METHODS

Materials. A medium-strength white flour (dough farinograph strength W < 260 x 10^{-4} J) from soft wheat was selected (BDS 602:1987) based on its specific volume (g/ml) and technological properties (Sofia Mel, Ltd.). Compressed yeast (Lesaffre Bulgaria, Ltd.), table salt, sucrose ("Sladeya", Zaharni Zavodi, Ltd.), margarine (Kaliakra, Flora Food Group, Ltd.), organic barley malt syrup (distributed by BioBalev, Ltd.), powdered milk ("Nido" instant full cream, Nestlé), pressed cake from defatted grape seeds (RAAB VITALFOOD GMBH, Germany), and grape seed flour (Vitis vinifera; 100% finely ground seeds; New S Net, Ltd.), from red grapes were used. **Standards and reagents.** To determine the total polyphenol content Folin-Ciocalteu reagent, gallic acid, sodium carbonate, sodium chloride, hydrochloric acid, acetic acid crystals, n-hexane, and ethanol were purchased. To assess the total antioxidant capacity of the bread samples via DPPH and FRAP assays, the following consumables were used: Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Steinheim, Germany). **Methods. Physicochemical measurements of the wheat flour (WF), grape seed cake (GSC), and grape seed flour (GSF):** crude protein (% d.w.) by total nitrogen using the Kjeldahl method; fiber (BDS 11374), fat (FERNÁNDEZ et al., 2010), moisture and ash (AOAC 1990). The wet gluten content in WF was determined according to BDS 754. **Dough preparation.** The basic formulation consisted of 100 g white flour (12.36% moist.), 1.8% fresh compressed yeast and 1.5% table salt (flour weight basis). The amount of water (30°C) for dough preparation was determined in farinograph units (FU), derived from the Brabender Farinograph (ICC Standard 115/1). The selected substitution levels of WF with GSC or GSF (flour weight based) were as follows: 5%, 7.5%, 10%, and 15%. After baking, the bread samples were left to cool at room temperature for 3 hours then sliced and stored in polyethylene bags for a week for the storage experiment. Another batch was kept at 22 ± 2°C for various time periods (1 to 3 and 5 days) to be frozen at -18°C after having reached each of the designated storage days. This was done in order to analyze the total polyphenol content (TPC) and overall antioxidant activity (AOA). **Physicochemical measurements of the bread samples:** weight, volume, specific volume (AACC International, 2010), crumb moisture (AACC, 1983, Method 44-1SA), water activity (Novasina EP-84), color (PCE–CSM 5), biochemical composition, TPC, AOA, total, plastic and elastic deformations (penetrometer AP–4/2) of the bread crumb as indicators of the shelf life. **Statistics.** Descriptive statistics (average values of each parameter, the standard error and the level of significance at p≤0.05). A multifactor ANOVA, Duncan test to find significance (p = 0.05) among storage time and the treatments on the deformation characteristics of the breads.

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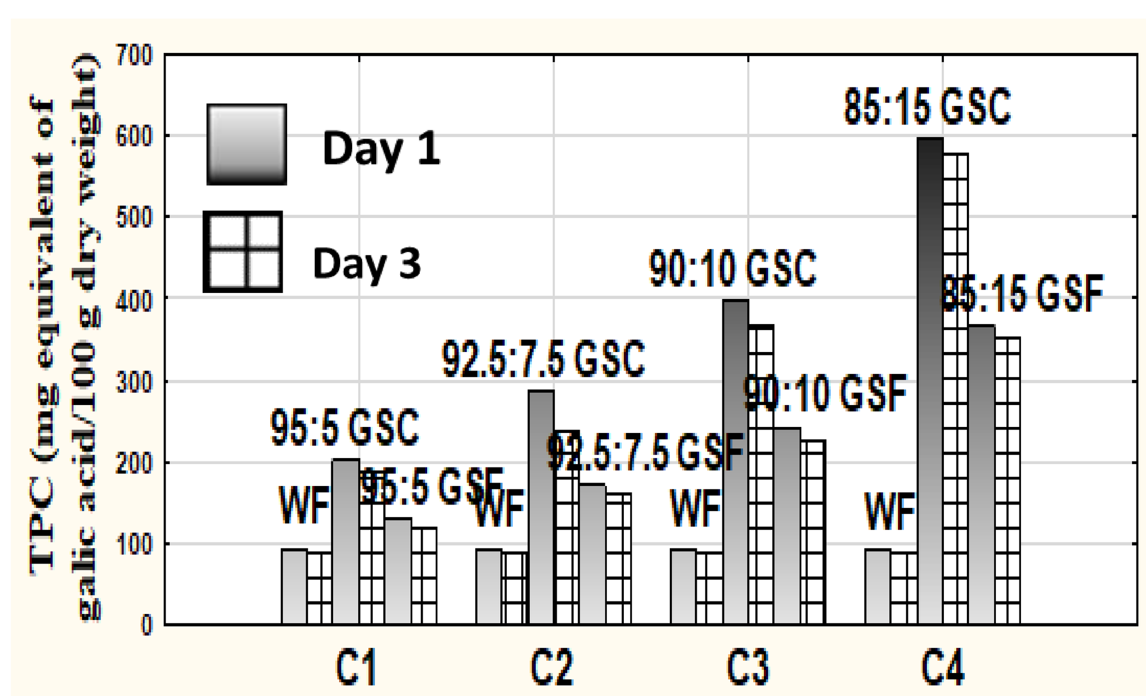
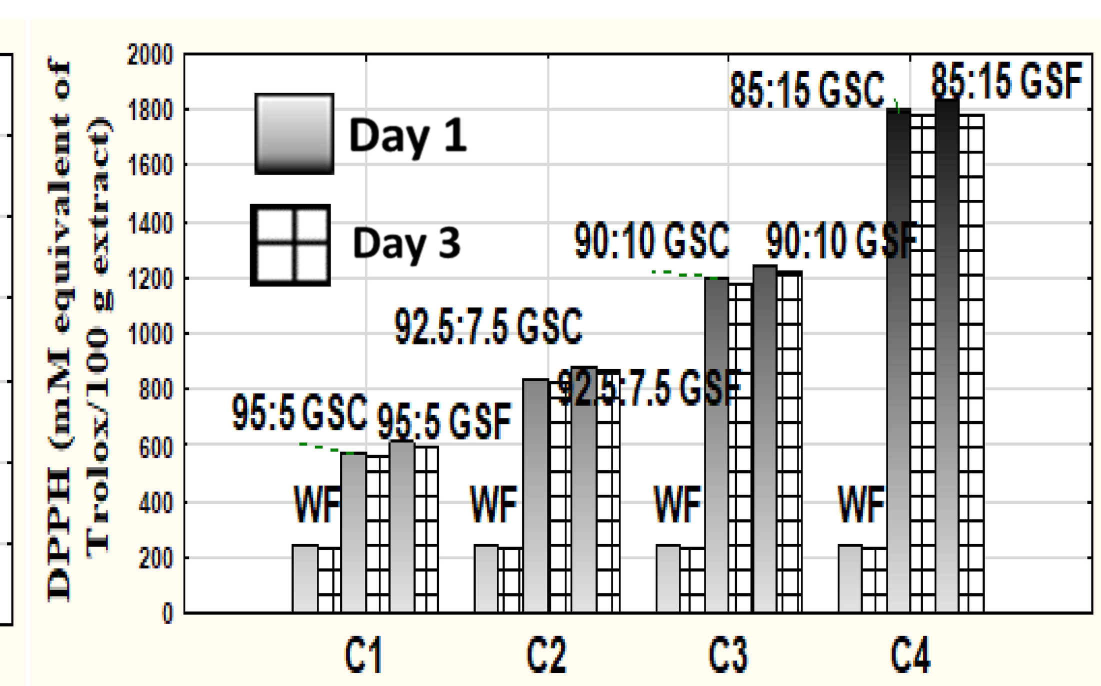
Table 1. **Instrumental analyses of the enriched bread and the control, p≤ 0.05**

Samples	WF:GSC/GSF, %	Specific volume*, g/cm3	Weight*, g	Crumb moisture*, %	L*	a*	b*	WI	Water activity(a_w)
C1	WF	3.94 a	398.30 a	37.38 a	69.74 a	2.25 a	14.47 a	30.65 a	0.872 a
	95 WF:5 GSC	4.17 b	418.21 c	42.50 b	51.68 b	8.86 b	16.45 b	13.74 b	0.873 a
	95 WF:5 GSF	4.13 b	396.97 b	35.19 c	54.58 b	5.35 c	10.90 c	18.09 c	0.868 b
C2	WF	3.93 a	399.10 a	37.09 a	69.25 a	2.58 a	14.18 a	33.96 a	0.876 a
	92.5 WF:7.5 GSC	4.02 b	427.44 c	44.20 b	41.76 b	8.17 b	16.50 b	8.33 b	0.871 a
	92.5 WF:7.5 GSF	3.79 a	395.27 b	31.01 c	52.13 c	6.30 c	11.15 c	16.14 c	0.875 a
C3	WF	3.94 a	398.10 a	36.89 a	68.85 a	2.32 a	14.85 a	34.59 a	0.875 a
	90 WF:10 GSC	3.45 b	437.91 c	47.10 b	41.94 b	8.97 b	17.17 b	8.23 b	0.887 b
	90 WF:10 GSF	4.39 c	391.70 b	37.92 c	46.93 b	6.87 c	11.25 c	12.68 c	0.875 a
C4	WF	3.93 a	398.5 a	37.12 a	69.52 a	2.15 a	14.55 a	33.84 a	0.874 a
	85 WF:15 GSC	2.87 b	426.39 c	44.30 b	32.01 b	10.17 b	16.31 b	4.82 b	0.877 a
	85 WF:15 GSF	3.92 a	401.37 b	37.85 c	38.60 c	6.69 c	11.00 c	8.37 c	0.876 a

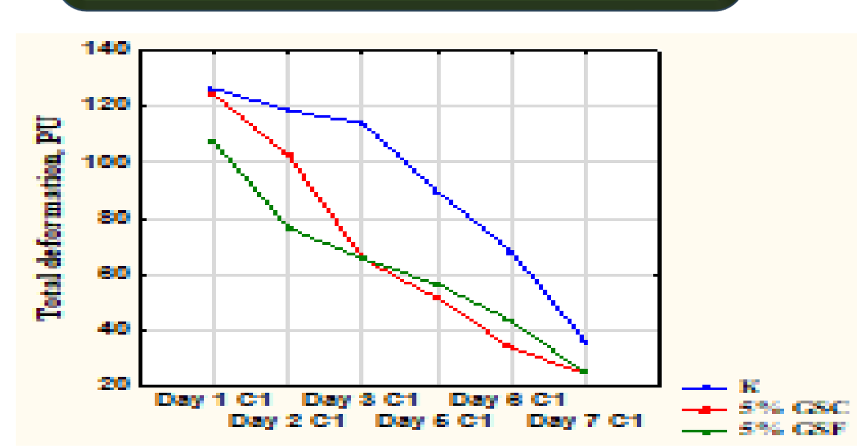
TPC of the control and samples, (p≤ 0.05), after storage in frozen state



AOA by DPPH of the control and samples, (p≤ 0.05), after storage in frozen state



Total deformation of the breads crumb, (p≤ 0.05)



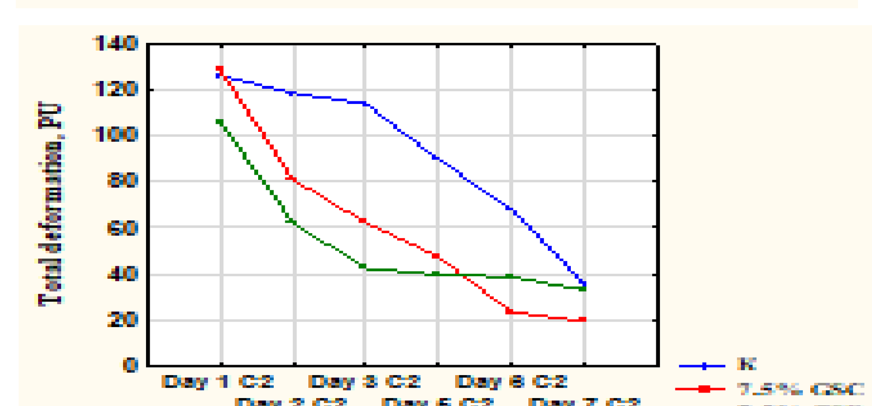


Figure 1. Control (C) and bread samples with added GSC (5%, 7.5%, 10%, and 15%) and GSF (5 and 7.5%)

RESULTS

It was found the reduced dough extensibility and weakening of the gluten network, resulting from the added fibers and non-gluten proteins, which probably decreased the dough's gas retention ability during final fermentation and baking, led to:

- Breads with greater weight and reduced specific volume, especially when 10% or 15% GSC was used;
- Significant increase (5% to 10%) in crumb moisture content in all samples containing GSC;
- Water activity values showed a narrow range of variation, with 5% GSF having the lowest values.

The inclusion of the additives resulted in:

- Significant change (p≤0.05) in crumb color intensity, with a trend towards darkening and browning, and the presence of the yellow hue in GSC samples, likely due to carotenoid pigments, which are known for their distinct yellowish tint.
- Enriched samples with significant increase of the antioxidant capacity, with total polyphenol content being 4 to 6.5 times higher and total antioxidant activity being 7 to 7.5 times higher compared to the control, when 15g of GSC or GSF was added per 100g of wheat flour. This increase was observed using both the DPPH and FRAP methods.

The comparison of all the formulations of enriched bread samples and the controls in terms of the average crumb moisture values demonstrated the aw values close to the threshold levels favorable for microbial growth: bacteria (aw≈0.90), yeasts (aw≈0.85-0.88), and molds (aw≈0.80), for both the controls and all the enriched samples.

Based on the commonly accepted 36 hours of shelf life of regular wheat bread and the deformation values reported in the literature, it was established these values were reached with 10% and 15% GSF after day 6; and with 5% and 10% GSC on day 5, in terms of total deformation (49 PU, according to literature).

From these data, it can be concluded that the new bread was fully comparable in quality to the control and retained its freshness for a sufficiently long period, on average between five and six days, which is entirely acceptable from the consumers' standpoint.