Extraction and characterization of functional and edible film-making properties for black cumin (N.sativa) meal proteins

Hilal KAYI, Yaren Buse BUDAK, Ahmet Yemenicioğlu

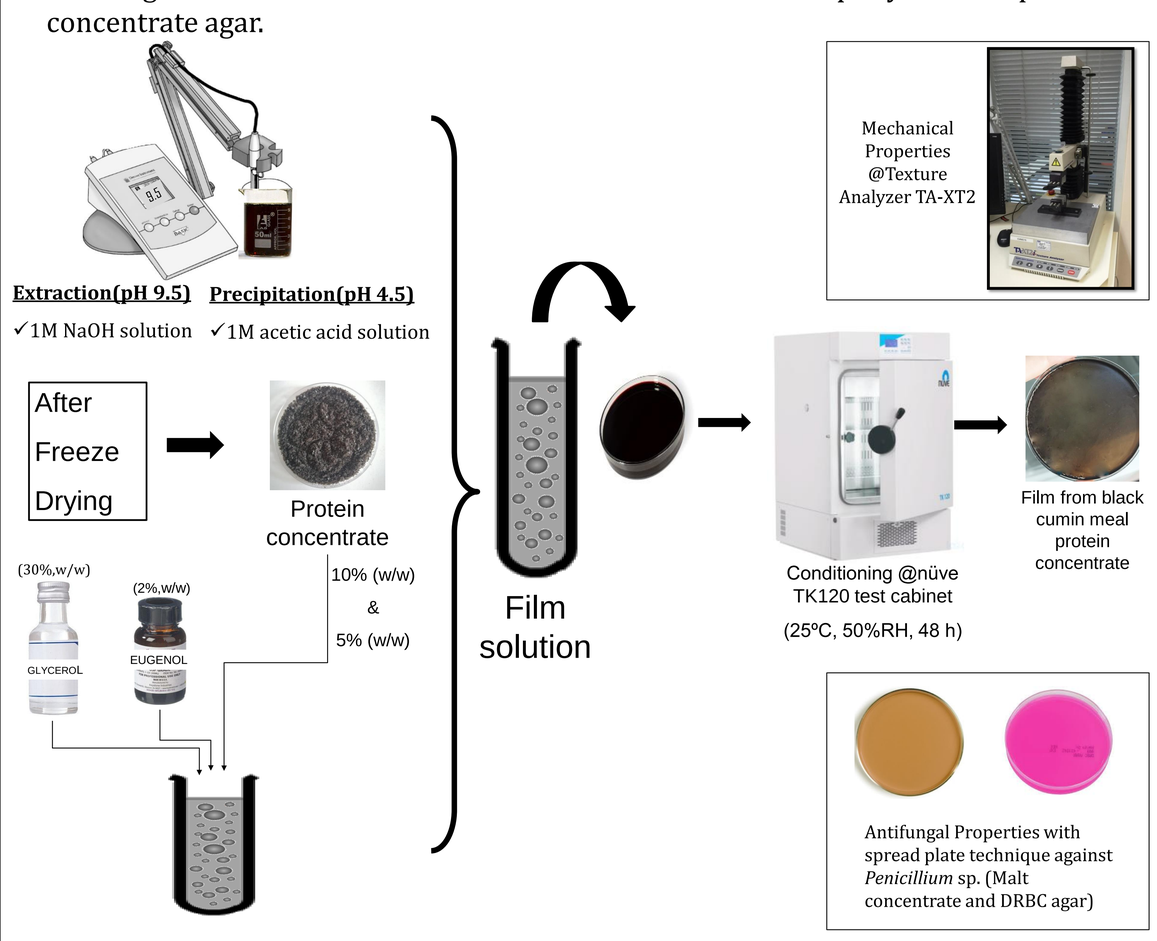
Department of Food Engineering, Faculty of Engineering, Izmır Institute of Technology, Izmir, Turkey

Abstract

The black cumin meal remained form cold-pressed oil extraction is a waste material that is used mostly as an animal feed. The aim of this project is to obtain and characterize the functional properties of protein concentrate of black cumin meal for the first time in the literature. The results obtained clearly showed that it is possible to obtain black cumin protein concentrate (BCC) with 56% protein content from black cumin waste with an initial protein content of 11%. The pH-solubility profile of BCC showed that the obtained proteins are soluble at around neutrality and at alkaline media between pH 6.5 and 9.5. The BCC obtained showed a concentration-dependent emulsion stabilizing effect at protein concentrations between 1 and 3%. The studies related to edible films of BCC clearly showed the good film forming capacity of this waste protein. The BCC films are flexible and showed elongation at break values between 150-280%. The films incorporated with EUG (2%) also showed antifungal activity on *Penicillium spp.* isolated from intentionally rotted bread. Thus, it seems that the antifungal BCC films could be employed in bakery products to extend their shelf life. This work clearly showed the possibility of evaluating black cumin waste as a value added protein concentrate. Further studies are needed to investigate nutritive value and potential food applications of isolated proteins.

Materials & Methods

- Extraction was done at pH 9.5 with two times isoelectric precipitation.
- Total protein content was determined by the Kjeldahl method using the conversion factor of 5.83 while soluble protein content determination was done with the Bradford method.
- Functional properties (water and oil holding capacity, emulsion and foaming capacity) analysis were done right after the preparation of protein concentrate.
- Film making properties of black cumin seed meal protein concentrate were examined with 10% and 5% protein solution with glycerol content (30%, w/w of BCC) and eugenol content (2%, w/w of film forming solution)
- Mechanical properties, solubility and swelling tests of films were done after 48 hour conditioning of films at 50% RH.
- Antifungal test was done with the isolated *Penicillium* sp by the help of malt concentrate agar.



Results & Discussion

Table 1. Protein content

Protein content(%)	
Black cumin meal	Black cumin protein concentrate
11,47	55,94

Table 2. Soluble protein content of black cumin concentrate

Soluble protein (g protein/g isolate)			
pH	Total protein (g protein/g isolate)	pH	Total protein (g protein/g isolate)
3	0,0271	6,5	0,4661 ± 0,0483
4,00	0,033	7	0,4660 ±0,0540
4,5	0,0199	8	0,5158 ± 0,0368
5,5	0,4941±0,0075	9,5	0,4964 ± 0,0760

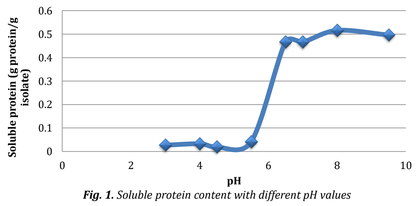


Fig. 1. Soluble protein content with different pH values

- Fig 1. shows that soluble protein content of lyophilizate at pH 8 slighlty higher than that at pH 9.5 which frequently used for concentration of plant based protein.

Table 3. Swelling degrees of films

SWELLING DEGREE %				
Sample/Time(min)	0	7, 5	15	30
control (10%)	0	1971,705±245,719[a]	1950,411±128,952[a]	2237,408±282,282[a]
%2 eug (10%)	0	1474,976±255,774[a]	1769,518±137,380[a]	1806,412±227,920[a]

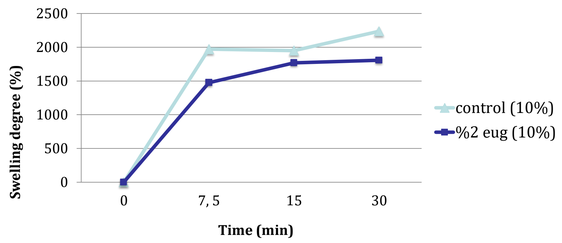


Fig. 2. Swelling values of films

- All films are swollen by absorbing a portion of water, instead of dissolving fully. Films from 10% black cumin concentrate showed less degree of swelling in both control and eugenol samples. It has been stated that the molecular properties of phenolic compounds can considerably affects the morphology and the strength of film matrix (Arcan& Yemenicioğlu,2011).
- In this study, swelling ratio of eugenol based films higher than control films which is also shown as Fig.2. Although there was significant difference among films according to time, there was no significant difference between each concentrations (p>0.05) .

Table 4. Mechanical properties of films

ID (7,5g-%30gly)	N Force	% Elongation	Mpa Tensile Strength	Mpa/% Young Modulus
10%pro	1,42 ± 0,18	284,16 ± 37,26	1,78 ± 0,22	0,06 ± 0,01
10%pro-%2eug	1,09 ± 0,18	186,33 ± 38,65	1,36 ± 0,23	0,07 ± 0,01
5%pro	0,34 ± 0,16	173,15 ± 64,11	0,43 ± 0,20	0,02 ± 0,01
5%pro-%2eug	0,60 ± 0,06	226,14 ± 31,17	0,60 ± 0,08	0,03 ± 0,01

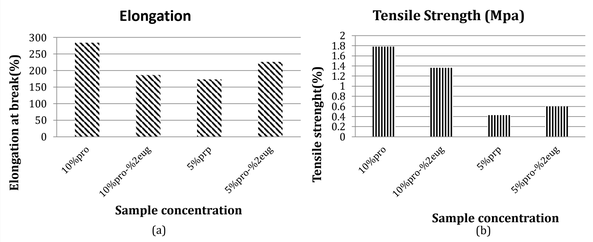


Fig. 3. (a)- Elongation of films; (b)- Tensile strength of films

- The elongation at break (E) value of 10% BCC films was significantly higher than that of 5% BCC film. The addition of EUG reduced the E value of 10% BCC film while a slight increase was observed in the E of EUG containing 5% BCC film. It seemed that the E is highly affected from protein/EUG ratio.
- The highest tensile strength (TS) was observed for control film with 10% BCC while this was followed by 10% BCC film with EUG, 5% BCC control film and 5% BCC film with EUG.
- These results showed that the EUG increased the brittleness of films, possibly due to extensive H-bond formation among associated BCC proteins.

Table 5. Emlsion capasities of black cumin seed meal protein concentrate

3%	%ES-30	%ES-1D	%ES-7D
black cumin meal Protein concentrate	100,00	84,47	79,45
2%	**%ES-30**	**%ES-1D**	**%ES-7D**
black cumin meal Protein concentrate	99,00	71,50	71,50
1%	**%ES-30**	**%ES-1D**	**%ES-7D**
black cumin meal Protein concentrate	97,00	67,00	67,00

Table 6. Moisture contents of films

SAMPLE	MOISTURE (%)	SOLUBILITY (%)
10%	7,077	84,06
10%+2%EUG	6,865	89,54
5%	6,627	100%
5%+2%EUG	8,776	100%

- It can be concluded that the increase of protein concentration caused a concentration-dependent increase in emulsion stability of BCC proteins (Table 5).
- The high dissolution rates of films shown in Table 6 indicated highly hydrophilic nature of BCC proteins.

Table 7. Antifungal activities of films on malt extract agar

2nd DAY		
PROTEIN CONTENT	EUGENOL CONTENT	ZONE AREAS(CM2)
10%	–	–
5%	–	–
10%	2%	554,35 ±141,71
5%	2%	–
3rd DAY		
PROTEIN CONTENT	EUGENOL CONTENT	ZONE AREAS(CM2)
10%	–	–
5%	–	–
10%	2%	106,48 ±18,31
5%	2%	–

- Zone formation against *Penicillium* sp. was observed only in films of 10% black cumin concentrate (BCC) with 2% eugenol (EUG). In contrast, films from 5% BCC did not form zones. This finding clearly showed that the film matrix should contain sufficient black cumin protein to retain and encapsulate volatile EUG within the films. The zones formed at the 2nd day got smaller at the 3rd day, and disappeared at the 4th day, since EUG in the films evaporated slowly during incubation (Significant differences in zones at 2nd and 3rd day were clearly observed at Table 7).

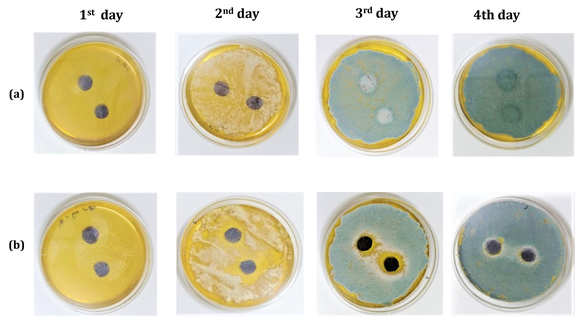


Fig. 4. (a) 10% film and (b) 10%+2%eug during 4 days of incubation at 25°C

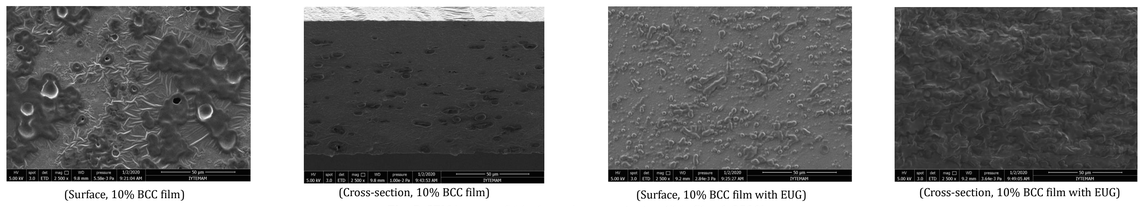


Fig. 5. SEM images of black cumin concentrate edible films(x2500)

- Fig. 5. SEM images of the films showed that the BCC film surface and depths of films contain some spherical pores. Rod-like fibers also exist within the film matrix. The addition of EUG caused some dramatic changes in film surface and cross-section morphologies. It appeared that the addition of EUG caused disappearance of spheric pores while it caused formation of a gritty appearance at the film cross-section,

Conclusion

In this study, a protein concentrate with 56% protein was obtained from black cumin waste remained from cold-press oil extraction process. The protein concentrate obtained was characterized for its pH-dependent solubility profile, ability to form edible films and emulsion stability. The results clearly proved that the BCC could be used for development of edible food packaging and coating materials and emulsifying agents in food systems. Further studies with edible films clearly showed that the BCC films with EUG could also be employed as antifungal coatings. The effectiveness of EUG loaded BCC films against *Penicillium* sp. isolated from intentionally rotted bread showed that the developed antimicrobial films could be used in bakery products to prolong their shelf-life.

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Hilal KAYI

Yaren Buse BUDAK